32725.1-LS-CF

Program

NANOFABRICATION AND BIOSYSTEMS: FRONTIERS AND CHALLENGES

May 8-12, 1994

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Professor Harvey Hoch
Cornell University
Geneva, NY

Conference Co-Chairs:
Professors Harold Craighead and Lynn Jelinski
Cornell University
Ithaca, NY

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13. ABSTRACT *(Maximum 200 words)*

The conference has two complementary themes. The first theme is the emerging trend in the application of micro- and nanofabricated devices to learn more about biological systems. The materials and physics research community has developed powerful and sophisticated methods of probing the electronic, magnetic and mechanical properties of materials on a micro scale. These techniques are beginning to be employed for biological measurements. For example, devices have been fabricated to selectively orient neurons, to elucidate how both fungal and mammalian cells perceive topographical signals, to record action potentials non-invasively from individual cells, and to determine what surface features promote cell and tissue compatibility to biomedical implants.

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The second complementary theme is the belief that there are important lessons to be gleaned from understanding and perhaps mimicking biological materials, and the ways that biological materials self-assemble. Biological molecules and systems have continually evolved over geological time scales to acquire properties that are highly attractive in today's drive for new engineering materials. Coupled with their tendency to self-assemble into highly organized two- and three-dimensional structures, biological materials make attractive targets for exploitation by materials scientists and engineers.

These two themes are symbiotic: microfabrication offers new tools for biology and medicine, while biology offers new materials and techniques for fabrication. Both of these themes underscore the need to encourage an early dialogue and partnership between biologists and physical scientists.

Co-sponsors

National Science Foundation
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Conference Objective

The objective of the conference is to explore contemporary and emerging approaches of nano- and microfabrication as they apply to biology and engineering. The intent of the conference is to bring together engineers/physical scientists and biologists/ biophysicists. The conference will address the materials science and engineering aspects of nano- and microfabrication, and how biologists have implemented such fabricated devices in their research endeavors. More specifically, the purpose of the conference will be to *i*) inform materials scientists and engineers about the needs of biologists, and to equally inform biologists about the possibilities of using nano and micro scale fabrication to unravel research problems not heretofore possible; *ii*) explore ways that materials scientists and engineers can exploit biological principles and biological assemblies to produce new and ever-smaller devices; and *iii*) initiate innovative, productive interactions between materials scientists, engineers, and biologists.

Conference Themes

The conference has two complementary themes. The first theme is the emerging trend in the application of micro- and nanofabricated devices to learn more about biological systems. The materials and physics research community has developed powerful and sophisticated methods of probing the electronic, magnetic and mechanical properties of materials on a micro scale. These techniques are beginning to be employed for biological measurements. For example, devices have been fabricated to selectively orient neurons, to elucidate how both fungal and mammalian cells perceive topographical signals, to record action potentials non-invasively from individual cells, and to determine what surface features promote cell and tissue compatibility to biomedical implants.

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Sunday, May 8, 1994

3:00 pm - 9:00 pm	Registration
6:00 pm	Dinner
7:30 pm	Social Hour with Poster and Video Session

Monday, May 9, 1994

7:00 am	Breakfast Buffet
8:30 am	Welcome and Opening Remarks Harvey Hoch, Conference Chair
	SESSION 1. MICROFABRICATION: CONTEMPORARY AND EMERGING TECHNIQUES Session Chair: Harold Craighead
	"Nanofabrication Techniques for Biological Systems: A Broad Overview" Evelyn Hu University of California, Santa Barbara
	"High Resolution Lithographic Techniques for Semiconductor Nanofabrication" Alfred Forchel Universität Wurzburg, Germany
10:00 am	Coffee Break
	"Principles of Material Etching" R. Germann IBM Rüschlikon, Switzerland
	"Ordered Growth of Semiconductor Materials" K. Ploog Max Planck Institüt, Stuttgart, Germany
12:00 noon	Lunch
1:30 pm	*Ad hoc* sessions/free time
6:00 pm	Dinner
7:30 pm	**SESSION 2. SCANNING PROBES AND MICROMECHANICAL DEVICES** Session Chair: Lynn Jelinski
	"Near-field Optical Characterization of Biosystems" R. Eric Betzig AT&T Bell Laboratories, Murray Hill, NJ
	"Biological Applications of Scanning Force Microscopy" Carlos Bustamante University of Oregon, Eugene
	"The Development and Applications of Micromechanical Devices in Biosystems" Kensall Wise University of Michigan, Ann Arbor
10:30 pm	Social Hour (cash bar)

Tuesday, May 10, 1994

7:00 am	Breakfast Buffet
8:30 am	**SESSION 3. DEVELOPMENT AND USE OF MICROFABRICATED INSTRUMENTS IN BIOLOGY AND MEDICINE** Session Chair: Harvey Hoch
	"Applications of Micro- and Nanofabrication to Single Cell Analysis" A.G. Ewing Pennsylvania State University, State College
	"Standard Test Targets for High Resolution Light Microscopy" Rudolf Oldenbourg and S. Inoue MBL, Massachusetts
10:00 am	Coffee Break
	"Bioselective Molecular Counting on the Ordered Protein Nano-array by AFM" Masuo Aizawa Tokyo Institute of Technology, Japan
	"Applications of the Cytosensor to Therapeutic Drug Discovery" H. McConnell Stanford University and Molecular Devices Corp., Stanford, California
12:00 noon	Lunch
1:30 pm	*Ad hoc* sessions/free time
6:00 pm	Dinner
7:30 pm	**SESSION 4. CELL AND TISSUE INTERACTIONS WITH MICROFABRICATED SURFACES** Session Chair: Harold Craighead
	"Cell and Neurone Growth Cone Behavior on Micropatterned Surfaces" P. Clark Imperial College, London
	"The Effects of Surface Topography of Implant Materials on Cell Behavior *in vitro* and *in vivo*" D. M. Brunette University of British Columbia, Vancouver
	"Analysis of Neuronal Migration on Microfabricated Surfaces" Philip Hockberger Northwestern University, Evanston, Illinois
10:30 pm	Social Hour (cash bar)

Wednesday, May 11, 1994

7:00 am	Breakfast Buffet
8:30 am	**SESSION 5. USE OF MICROFABRICATED DEVICES TO STUDY CELL FUNCTION AND DEVELOPMENT** Session Chair: Lynn Jelinski
	"Micro-control of Neuronal Outgrowth" Helen Buettner Rutgers University, New Jersey
	"Biofunctionalized Membranes on Solid Surfaces" Robert Tampé and Erich Sackmann Max-Planck-Institüt, Martinsried, and Technical Universität Munich, Germany
10:00 am	Coffee Break
	"Formation of a Simplified Brain on Microfabricated Electrode Arrays" A. Kawana NTT, Japan
	"Microfabricated Surfaces in Signaling for Cell Differentiation in Fungi" H.C. Hoch Cornell University, Geneva, New York
12:00 noon	Lunch
1:30 pm	*Ad hoc* sessions/free time
6:00 pm	Dinner
7:30 pm	**SESSION 6. BIOLOGICAL PROCESSES AS TOOLS AND PROBLEMS APPLICABLE TO NANOFABRICATION** Session Chair: Harvey Hoch
	"Nanoscale Biomechanics" Robert Campbell U.S. Army Research Office, Research Triangle Park, North Carolina (15 minute presentation)
	"Contemporary Problems in Biology: Contractile Materials" G. H. Pollack University of Washington, Seattle
	"Force Generation by the Microtubule Based Motor Protein Kinesin" F. Gittes and J. Howard University of Washington, Seattle
	"Microsensors and Microstructures for Biomedical Applications" M. Esashi Tohoku University, Japan

"Confocal Imaging of Flows in Microvessels"
Mary Lowe and Darren Hitt
Loyola College, Baltimore, Maryland
(15 minute presentation)

"Nanodesigning in Biological Composites"
Mehmet Sarikaya
University of Washington, Seattle
(15 minute presentation)

10:30 pm — Social Hour (cash bar)

Thursday, May 12, 1994

7:00 am	Breakfast Buffet
8:30 am	**SESSION 7. APPLICATIONS OF MICROFABRICATION IN MOLECULAR BIOLOGY** Session Chair: Harvey Hoch "Technology Needs for the Human Genome Project" David Burke University of Michigan, Ann Arbor "Use of Micromachined Structures for the Manipulation of Biological Objects" Masao Washizu Sekei University, Tokyo, Japan
10:00 am	Coffee Break "BIAcore: A Surface Plasmon Biosensor for Characterization of Biospecific Interactions" M. Malmqvist Pharmacia, Uppsala, Sweden "Nanoscale Structures Engineered by Molecular Self-Assembly of Functionalized Monolayers" D. Allara Pennsylvania State University, State College
12:00 noon	Lunch and Open Discussion with Panel "New Directions and Applications of Microfabrication for Biology (and Biology for Microfabrication)" Panel Leaders: Harold Craighead and Lynn Jelinski Cornell University, Ithaca, New York
2:30 pm	Adjournment

Abstracts

NANOFABRICATION AND BIOSYSTEMS: FRONTIERS AND CHALLENGES

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May 8-12, 1994

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MONDAY, MAY 9, 1994

SESSION 1
MICROFABRICATION: CONTEMPORARY AND EMERGING TECHNIQUES
Session Chair: Harold Craighead

"Nanofabrication Techniques for Biological Systems: A Broad Overview"
Evelyn Hu
University of California, Santa Barbara

"High Resolution Lithographic Techniques for Semiconductor Nanofabrication"
Alfred Forchel
Universität Wurzburg, Germany

"Principles of Material Etching"
R. Germann
IBM Rüschlikon, Switzerland

"Ordered Growth of Semiconductor Materials"
K. Ploog
Max Planck Institüt, Stuttgart, Germany

Nanofabrication Techniques for Biological Systems: A Broad Overview

Evelyn L. Hu
Department of Electrical and Computer Engineering
University of California
Santa Barbara, CA 93106

Fabrication techniques have been developed that allow access to size scales commensurate with biological systems, allowing the formation of structures and probes that may facilitate the study of those systems. Similarly, biologiccal principles of organization and interaction offer promise for extending fabrication techniques to even smaller size scales, with high degrees of selectivity, and uniformity. This talk will provide a broad overview, and an introduction to the methodology of fabrication that has been developed largely for silicon integrated circuits. The approach is basically a planar one, forming 3-dimensional patterns through a series of 2-dimensional patterning steps. The kinds of lithographies that can be used to form the patterns will be briefly discussed, to be amplified further in Professor Forchel's talk. Pattern transfer techniques, in particularly the various gas-phase, high resolution etching techniques will be introduced, with further details to be provided by Dr. Germann. The utilization of such fabricated structures in biological experiments will be illustrated; for example, experiments carried out by Professor C.D.W. Wilkinson, and co-workers at the University of Glasgow, have examined cell motility, and alignment using simply fabricated structures.

Recent innovations in fabrication have been associated with Micro-Electro-Mechanical Structures (MEMS), where the techniques developed for planar patterning have now been extended to create truly three-dimensional components such as cantilevers, rotating disks, micromotors and tweezers. Examples of fabrication strategies for MEMS will be given: these are the same approaches that are utilized in the formation of high resolution tips for Scanning Tunneling Microscopes and Atomic Force Microscopes. Sensors based on such MEMS are already being incorporated into commercial applications (auto safety, for example), but their full potential is only beginning to be realized, and there may be advantages in utilizing mechanically active fabricated structures in biological studies.

Finally, research in nanostructures and their fabrication have revealed a number of limitations in the current nanofabrication techniques, with respect to achievable resolution, uniformity of arrays of nanostructures, low process-associated damage and control of nanofabricated surfaces. Researchers are already looking towards self-organizing growth mechanisms of inorganic materials, and more sensitive exploration of chemical treatment at monolayer scales, in order to control surfaces. Biological systems, with self-organizing, highly selective reactivities, may therefore provide much insight and direction in developing the next generation of nanofabrication techniques.

High Resolution Lithographic Techniques for Semiconductor Nanofabrication

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Technische Physik
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Semiconductor structures with dimensions on the order of the de Broglie wavelength allow to control the electronic levels of the materials via the vertical or lateral size of the structures. By using patterns with widths in the s 20 nm range the emission energy of a given semiconductor may be varied locally on a single chip due to lateral quantization effects in one dimensional (quantum wire) and zero dimensional (quantum dot) structures. In order to fabricate semiconductor structures with sizes on the 20 nm range high resolution electron beam lithography is predominantly used. In order to define ultranarrow structures reliably the proximity effect, i. e. the unintentional exposure of one part of a pattern by other parts, has to be suppressed. This may be achieved by using very high electron energies (r 100 keV), which give rise to a very widely extending weak background exposure by backscattered electrons. Recently, low voltage (s 5 keV) exposures have been investigated based on field emission electron sources. In this case backscattering from the semiconductor substrate into the organic resist layer may be completely supressed. The paper reviews low and high energy exposure techniques for the definition of structures with widths in the s 20 nm range. The most important criterium for the use of these techniques to define lateral quantum structures is the control of width fluctuations. Fluctuations arise from the size of the polymer chains used as a resist. By using electron beam lithography and wet chemical etching quantum wire and quantum dot structures have been defined on different III - V semiconductor systems and will be presented at the meeting. The lateral confinement in InGaAs/InP quantum wires with widths down to 8 nm leads to an increase of the band gap of about 80 meV. If the quantum wire and dot structures are excited with high laser power, the emission clearly displays transitions within up to three lateral subbands. By model calculations the lateral quantization effects observed in nanostructures can be related directly to the measured pattern dimensions.

Principles of Materials Etching

R. Germann
IBM Research Division
Zurich Research Laboratory
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One approach for the fabrication of micro- and nanostructures is the definition of a mask pattern with high-resolution lithography and the subsequent transfer of this pattern into the underlying substrate by means of etching. The etching process can be done isotropic etching characteristics and limits this approach to very shallow etch depths or to certain crystal directions, where WCE can be anisotropic in special cases.

Dry etching methods based on low-pressure plasmas of inert and/or reactive gases are much better suited for the anisotropic and controlled etching of a variety of materials. Ions with typical energies of up to a few hundred electron volts, radicals and reactive neutrals can interplay through physical and chemical mechanisms and lead to anisotropic, selective, controllable and reproducible etching.

In the past twenty years, a great variety of dry etching methods has been developed based on different types of reactors, gases, pressures ranges and excitation frequencies. This talk will review the various dry etching methods and classify them according to the underlying etching mechanism. Dry etching will be illustrated by a few specific examples of III/V semiconductor materials (like AlGaAs and InP-based systems) in the area of nanostructures and optoelectronics.

One of the major problems of ion assisted etching is the etching induced damage which is mainly caused by ions with a small but finite energy. This talk will give examples of the characterization of ion-induced damage and discuss ways to overcome it.

Ordered Growth of Semiconductor Materials

Klaus H. Ploog
Paul-Drude-Institut für Festkörperelektronik
D-10117 Berlin, Germany

Ordered crystal growth in terms of spontaneous structural ordering on a sublattice has been observed in several ternary and quaternary III-V semiconductor alloys. The ternary $Ga_XIn_{1-X}P$ (x=0.5), for example, exhibits CuPt-type structural sublattices, and a strong correlation between the degree of ordering and the electronic properties exists. In this paper we present new phenomena of ordered growth. We discuss the formation of GaAs quantum wires and quantum dot structures in an AlAs matrix which relies on the natural evolution of nanometer-scale periodic corrugations on (311)-, (211)- and (111)-oriented GaAs surfaces combined with a unique phase shift of these corrugations during AlAs/GaAs heteroepitaxy. The effect of strain on height and periodicity of the surface corrugations and the feasibility of step bunching provide a unique means to tune the dimensions of the quantum wire and quantum dot structures. These new phenomena of ordered growth described here can be understood in terms of self-organization, i.e. the ability of the system comprising many units and subject to constraints to organize itself in various spatial and temporal activities.

MONDAY, MAY 9, 1994

SESSION 2
SCANNING PROBES AND MICROMECHANICAL DEVICES
Session Chair: Lynn Jelinski

"Near-field Optical Characterization of Biosystems"
R. Eric Betzig
AT&T Bell Laboratories, Murray Hill, NJ

"Biological Applications of Scanning Force Microscopy"
Carlos Bustamante
University of Oregon, Eugene

"The Development and Applications of Micromechanical Devices in Biosystems"
Kensall Wise
University of Michigan, Ann Arbor

Near-field Optical Characterization of Biosystems

Eric Betzig
AT&T Bell Laboratories
600 Mountain Ave.
Murray Hill, NJ 07974

Near-field scanning optical microscopy (NSOM) exploits the optical interaction arising from a sub-wavelength sized probe in close proximity to a sample in order to image the surface on a scale well beyond the conventional diffraction limit. Potential applications include materials characterization, inspection of semiconductor devices, and high density data storage. However, because it combines high resolution with non-invasiveness and versatile contrast, NSOM may be particularly well suited to the investigation of biosystems. After an introduction to the relevant technology, several preliminary examples will be discussed, including absorption imaging of stained tissue sections, fluorescence imaging of cytoskeletal actin within whole fixed cells, and double label imaging of lipid domains and immunoreceptors within the cell membrane. Recent efforts involving the detection and characterization of single chromophore dye molecules will also be presented. Finally, possible future directions such as dynamic imaging of living cells and molecular resolution microscopy/spectroscopy will be discussed, with emphasis on the hurdles which must be overcome in each case.

Biological Applications of Scanning Force Microscopy

Carlos Bustamante
Institute of Molecular Biology,
University of Oregon, Eugene, Oregon 97403

Invented in 1986[1] the Scanning force Microscope (SFM) uses a sensor tip carried by a flexible cantilever to touch and characterize the topography of a surface. Gerd Binning and Calvin Quate were the first to realize that while the force constant between two atoms is about 10 N/m, a cantilever made up of a piece of household aluminum foil 4 mm long and 1 mm wide has a force constant of only about 1 N/m. [2] Thus, it is relatively easy to build a cantilever that would bend or deflect rather than scratch the surface, and an image of the sample can be formed by recording the deflections of the tip-carrying cantilever as it scans over the sample. At present, the resolution of the SFM on biological samples is slightly below that of the transmission electron microscope, i.e., about 50-80 Å, but this is likely to improve through the use of sharper tips. Moreover, the SFM has the capability to image macromolecules under aqueous environments.[3] A summary of the most recent developments in the applications of SFM will be presented.[4] Recent images of macromolecular assemblies imaged by various groups will be used to illustrate the present capabilities of SFM in biology. Results obtained on a variety of protein-DNA complexes will be reviewed, with particular emphasis on the new information that is emerging from these SFM studies.[5] One particular case is that of chromatin in vertebrates. This is a structure that has received a great deal of attention by electron microscopic techniques. Results of experiments performed on chicken erythrocyte chromatin and obtained in the newly developed *tapping* mode will be presented. These results will be used to illustrate the potential of SFM as a method to investigate biological systems at high resolution yet in conditions that largely preserve native structures. Recently obtained images of transcription complexes in saline buffers will also be presented. These results represent a step towards realizing the potential of SFM to monitor and follow the dynamics of molecular assembly processes in almost real time.

Finally, current efforts to develop sharper tips as a way to improve the present limitation in spatial resolution of the instrument will be described.

Bibliography:

1. G. Binning, C. F. Quate and Ch. Gerber, *Phys.. Rev. Lett.*, 1986, **56**: 930.
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3. Drake B. et al., *Science* 1989, **243:** 1586-1589.
4. C. Bustamante, D. Keller and G. Yang, *Curr. Opi. Str. Biol.* 1993, **3**: 363.
5. W. Rees, R. Keller, J. Vesenka and C. Bustamante, *Science* 1993, **260**: 1646.

The Development and Application of Micromechanical Devices in Biosystems

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Center for Integrated Sensors and Circuits
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During the past few years, substantial progress has been made in the development of integrated microelectromechanical devices for use in biological systems. Combining sensors, microactuators, and microelectronics on a single chip or in highly-integrated multi-chip modules, these devices are potentially capable of monitoring a wide range of physical variables with unprecedented accuracy and of controlling events with a spatial resolution extending down to the cellular level. Biosystems represent one of the most important application areas for these devices, and yet the problems in such systems are particularly challenging. This paper will discuss some of the problems that have recently been solved as well as several that still remain.

The paper first reviews the technologies that are currently available and the microstructures that can be formed using them. Such structures are based on bulk and surface micromachining, wafer-to-wafer bonding, and electroforming processes such as LIGA. While a wide range of microstructures having moving parts have been demonstrated in the laboratory, including microvalves and micropumps, difficult challenges remain in their application to biosystems, especially in the development of hermetic packages that allow selective access to the biosystem and promote acceptance of the microsystem by the tissue.

Some of the recent successes and remaining challenges are illustrated by three emerging devices. First, an active micromachined pressure-sensing catheter is described. This device allows multipoint pressure measurements in small vessels such as the coronary arteries of the heart with a pressure resolution of 1mmHg and a width of only 0.35 mm. Hermetic feedthrough technology allows these devices to be vacuum-sealed at the wafer level. The device operates using only two leads, simplifying packaging and representing a first step in introducing more sophisticated diagnostics into catheter-based instruments. A second illustration is provided by the development of a family of multichannel recording and stimulating electrode arrays for use in studies of information processing in biological neural networks and in neural prostheses. These devices can be formed in two- and three-dimensional arrays having site spacings of typically 100-200 μm and displace less than one percent of the surrounding tissue. They are facilitating important new studies of neural systems and now permit chronic studies to be performed routinely for periods of many months. Currently, they are also being extended to enable thermal and chemical interactions at the cellular level. Finally, work on hermetically-sealed microtelemetry systems will be mentioned that should soon permit such devices to be implanted with no external leads.

TUESDAY, MAY 10, 1994

SESSION 3
DEVELOPMENT AND USE OF MICROFABRICATED INSTRUMENTS IN BIOLOGY AND MEDICINE

Session Chair: Harvey Hoch

"Applications of Micro- and Nanofabrication to Single Cell Analysis"
A.G. Ewing
Pennsylvania State University, State College

"Standard Test Targets for High Resolution Light Microscopy"
Rudolf Oldenbourg and S. Inoue
MBL, Massachusetts

"Bioselective Molecular Counting on the Ordered Protein Nano-array by AFM"
Masuo Aizawa
Tokyo Institute of Technology, Japan

"Applications of the Cytosensor to Therapeutic Drug Discovery"
H. McConnell
Stanford University and Molecular Devices Corp., Stanford, California

Applications of Micro- and Nanofabrication to Single Cell Analysis

Andrew G. Ewing
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152 Davey Laboratory
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Our research efforts concern development and application of chemical sensors for analysis in and at single cells of the nervous and immune systems. Chemical methods to separate picoliter and femtoliter samples by capillary electrophoresis have been developed. In addition, electrochemical sensors with micrometer to nanometer tip dimensions have been used to monitor chemical dynamics at single cells. In fact, these methods are sensitive and spatially selective enough to monitor neurotransmitter release for single exocytotic events.

Three major challenges for the analysis of single cells are 1) development of methodology to detect a wider array of chemical species, 2) development of selective methods to monitor chemical dynamics, and 3) development of electrodes small enough to place in single synapses. The first challenge can be met by use of rapid derivatization reagents and fluorescence detection in capillary electrophoresis. However, derivatization of the contents of a single cell requires an extremely small reaction vial to minimize dilution and to maintain rapid reaction kinetics. We have lithographically fabricated arrays of microvials having volumes ranging from 8.5 to 95 picoliters. These vials have been used to carry out derivatization reactions on picoliter samples with subsequent separation by capillary electrophoresis. In addition, these picoliter vials should be useful for sampling substances released by nerve cells into the extracellular fluid while again minimizing dilution.

The second challenge is to develop selective methods to monitor chemical dynamics. Continuous free zone electrophoresis in narrow channels has been demonstrated. This new technique has the ability to continuously sample and separate analytes from picoliter microenvironments. This is achieved when one end of a small bore capillary is placed in the environment of interest and material is continuously sampled via electromigration into the entrance of a quartz channel which has an internal height and width of 48 m and 2 cm, respectively. By moving the capillary across the entrance of the channel, the sampled material is continuously injected into the channel. Analytes are then electrophoretically separated as they migrate the 5 cm length of the channel. As the separated analytes emerge from the channel, they are detected in a manner in which spatial resolution is preserved. Laser induced fluorescence utilizing fiber optic arrays and electrochemical detection with a multi-electrode array are two detection schemes which can be employed. An array of 100 electrodes 95 m wide and 5 m spacing has been fabricated as a spatial detector. The eventual goal of this work is to provide a method capable of selectively monitoring single quantal events at nerve cells.

Development of electrodes small enough to place in single synapses is the third major challenge and we are still working on electrode fabrication techniques. Electrodes with total tip diameters as small as 400 nm have been developed; however, to successfully place an electrode in a synapse, it will have to possess a tip diameter of approximately 50 nm.

Standard Test Targets for High Resolution Light Microscopy

Rudolf Oldenbourg and Shinya Inoué
Marine Biological Laboratory
Woods Hole, MA 02543, USA

The light microscope, aided by analog and digital image enhancement, is now used to visualize objects, and measure events, at dimensions considerably below the Abbe limit of resolution. This includes the third dimension along the microscope axis. Therefore, it is increasingly important that we assess experimentally and understand quantitatively images formed by high resolution microscope optics from simple, well characterized test objects. This is necessary both to avoid misinterpreting images and to gain further insight into the specimen fine structure. To address this problem, we are developing and fabricating test slides for the following two purposes: (A) to improve our understanding of the optical transfer functions and reliability of the image generated in three dimensions by wide field and confocal microscope optics in various contrast modes; (B) to provide a standard for assessing, and to help improve, the quality of microscope optics, electronic imaging equipment and digital image processors.

The test targets which we have fabricated contain line gratings with reliable spacings down to 100 nm. The gratings allow, for the first time, direct measurement of contrast transfer characteristics of high resolution microscope objectives with numerical apertures of up to 1.4. The gratings extend in planes that are either perpendicular, parallel or at some skewed angle to the microscope axis, thus allowing one to assess the 3-D imaging characteristics of high resolution microscope optics. We have fabricated test targets for different contrast modes including transmission and reflection microscopy (metallic targets), phase contrast, polarizing and differential interference microscopy (phase targets made of quartz) and fluorescence microscopy (targets made of fluorescently doped resist). We plan to report on the use of these test targets to the study of 3-D imaging.

The test targets were fabricated in collaboration with the National Nanofabrication Facility at Cornell University, using electron lithography or focused ion beam techniques. All test targets were fabricated using standard microscope coverglass, selected for 0.17 mm thickness (required for optimum correction of high NA objective lenses), as substrates. In a recent study we have used the test targets to measure the contrast transfer characteristics of confocal and non-confocal microscopes (Oldenbourg et al., 1993). The project is supported in part by the National Institutes of Health grant R-37 GM31617 and National Science Foundation grant DCB-8908169 awarded to S.I., and National Institutes of Health grant R01 GM49210 awarded to R.O. The National Nanofabrication Facility is supported by the National Science Foundation under grant # ECS-8619049, Cornell University, and industrial affiliates.

References:

Oldenbourg, R., H. Terada, R. Tiberio and S. Inoué. 1993. Image sharpness and contrast transfer in coherent confocal microscopy. *J. Microsc.* 172:31-39.

Bioselective Molecular Counting on the Ordered Protein Nano-Array by AFM

M. Aizawa, K. Nishiguchi, E. Kobatake, and Y. Ikariyama
Department of Bioengineering
Tokyo Institute of Technology
Nagatsuta, Midori-ku
Yokohama 227
Japan

Biosensing technology has made a marked progress specifically in this decade with a great success in implementation of biological selectivity into electronic and optoelectronic devices. One of the ultimate goals of biosensing technology may be to emerge a new technology to quantitate selectively a specific molecule in a specific site.

We have succeeded in fabricating an ordered array of antibody on the solid surface and quantitating selectively the corresponding antigen molecules on the antibody array by atomic force microscopy (AFM). Protein A (Prot A), which has a specific binding affinity to the Fc part of antibody, was found to form a monolayer on the water surface. A monolayer film of Prot A was deposited on the solid surface by LB film technique. Antiferritin antibody was then self-assembled on the Prot A layer. Each protein layer was characterized in ordered structure by AFM. In the further step, ferritin was self-assembled on the antibody array. Ferritin molecules self-assembled on the antibody nano-array was quantitated by AFM.

Applications of the Cytosensor™ to Therapeutic Drug Discovery

Harden M. McConnell
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Research workers at Molecular Devices Corporation have discovered that the receptor-mediated triggering of biological cells can be detected by measuring associated changes in the production of acid. The rate of acid production is monitored using a silicon based light-addressable potentiometric sensor (LAPS), a surface of which is pH sensitive. The commercial version of this device, the Cytosensor™, provides a means for maintaining cells in close proximity to the silicon sensing surface, and at the same time provides a fluid flow-through system to introduce fresh medium. Biological cells that are receptor positive for a specific receptor ligand are triggered when this ligand is introduced by a valve switch into the medium flowing over the cells, and biochemical response is then detected by a change in the rate that the cells acidify the medium.

The Cytosensor™ and its contemplated extensions offer an efficient approach to screen for new therapeutic drugs, and natural receptor ligands, especially ligands for the orphan receptors whose genes are now being sequenced in the human genome sequencing efforts.

In discussions of Cytosensor™ applications it is sometimes convenient to draw a rough analogy with modern computers. Computers can be used to solve a wide variety of problems by using charge carriers (electrons) in a hardware system that is coupled with appropriate software. Likewise, the Cytosensor™ can be used to solve a wide variety of problems by using charge carriers (protons) in a hardware system coupled with appropriate liveware. In the latter case the liveware is realized by the selection and genetic manipulation of biological cells.

TUESDAY, MAY 10, 1994

SESSION 4
CELL AND TISSUE INTERACTIONS WITH MICROFABRICATED SURFACES
Session Chair: Harold Craighead

"Cell and Neurone Growth Cone Behavior on Micropatterned Surfaces"
P. Clark
Imperial College, London

"The Effects of Surface Topography of Implant Materials on Cell Behavior *in vitro* and *in vivo*"
D. M. Brunette
University of British Columbia, Vancouver

"Analysis of Neuronal Migration on Microfabricated Surfaces"
Philip Hockberger
Northwestern University, Evanston, Illinois

Cell and Neurone Growth Cone Behaviour on Micropatterned Surfaces

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Microfabrication technologies provide opportunities for bioscientists to examine how cells interact with, and their behaviour controlled by, their local microenvironment. Such techniques have been employed to fabricate micropatterned topographies, and micropatterns of differential adhesiveness, to examine the responses of cells and growth cones. The topographies used include single steps and multiple parallel grooved substrata of micrometre dimensions made using conventional photolithography, and ultrafine multiple parallel grooved substrata (sub-micrometre dimensions) patterned using laser interferometry. The reactions of cells cultured on these substrata suggest that cells and growth cones respond probablistically, and that cell type, feature magnitude (ie. step height and groove depth) and feature density (ie. pattern repeat) strongly influence the degree of response. Increased magnitude and increased feature density (ie. decreased repeat distance) resulted in increased alignment and elongation of fibroblastic and epithelial cells, and outgrowth of neuritic processes from neurones. Using conventional photolithography and organosilane coupling to glass, patterns of adhesiveness were made. Cells were cultured on multiple parallel repeat patterns of alternating adhesive/non-adhesive tracks of various periods. Their responses (alignment and elongation of cells, and guided neurite outgrowth) were dependent on the packing density (ie. repeat period) of the patterns. The smaller the period, the smaller the effect. Single narrow cues did precisely guide neurite elongation. The differences in the effects of cue density on the responses of cells to topography or patterned adhesiveness, suggest very different, indeed opposing, effects on cell behaviour. Closely spaced topographic features reinforce each others effects, whereas cells are able to bridge narrow non-adhesive regions thereby over-riding the guidance cues. Adhesive guidance allows more precise control if these geometrical constraints are considered. These different effects have important implications in vivo in development and regeneration, and will influence the design of materials and devices destined to be in contact with tissues.

The Effects of Surface Topography of Implant Materials on Cell Behaviour *in vitro* and *in vivo*

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The reaction of cells to the topography of the substratum to which they are attached was one of the first phenomenon observed in tissue culture by Ross Harrison in 1911, and subsequent studies have shown that surface topography is an important factor in controlling the shape, orientation and adhesion of mammalian cells. Surface topography also exerts significant effects in vivo where the cells interact with the specific topographies inherent in other cells and extracellular matrix. In particular one effect of surface topography, topographic guidance, has been postulated as being involved in diverse processes in vivo including development, pathogenesis, and repair and regeneration after wounding. Micro and nanofabrication techniques can be used to prepare substratum that can be used in investigations that explore the basic mechanisms of cell behaviour in response to the topography of their substratum as well as in more applied studies in which topography on implants is altered to produce desired cell responses. As an example of the application of microfabricated materials to basic biological questions, data will be presented showing that grooved substrata produced by micromachining can alter cell shape, distribution of cytoskeletal elements, and gene expression. The possibility of improving implant performance by altering surface topography will be illustrated by data showing that grooved substrata replicated onto the surface of a percutaneous device can inhibit epithelial downgrowth, a process which is a major problem with these devices. Besides topographic guidance, surface topography on implanted devices might be modified so as to accomplish desired goals such as 1) the selection of specific cell populations attached to the implant through differential effects on cell adhesion, 2) the modification of the functions of cells attached to the implant through control of their shape and polarity, 3) the organization of tissues adjacent to the implant, and 4) the creation of designer microenvironments where desired cell interactions can occur. As an example of the ability of micromachined surfaces to enhance specific cell processes, data will be presented to show that a pitted surface produced by micromachining leads to increased bone-like tissue production on subcutaneous implants in rats. Taken together these data indicate that microfabricated surfaces have considerable ability to modify cell behaviour, and it would be expected that micro- and nano-fabrication techniques will prove to be powerful tools in the engineering of biomaterial surfaces for specific purposes.

Analysis of Neuronal Migration on Microfabricated Surfaces

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Developing neurons produce neurites with growth cones that are capable of navigating through complex environments to locate distant target cells. There is substantial evidence that growth cones use extracellular pathways, comprised of both topographical and chemical cues, to find their targets. We are using microfabrication techniques to create well-defined, chemically-patterned surfaces upon which to study neuronal pathfinding and migration in culture.

Surfaces are prepared using silane coupling chemistry and photolithography [1]. Recently we modified this method to reduce production time and cost, and made it feasible in a standard biological laboratory [2, 3]. Briefly, glass coverslips were spin-coated with photoresist and exposed to UV light through a mask. After removing exposed photoresist, alkylsilanes (dimethylsilane, hexadecylsilane or octadecyldimethylsilane) were bound to the exposed regions. The remaining pattern of photoresist was then removed, and aminosilanes (ethylenediamine-propylsilane) were bound to the newly exposed glass regions. This process yields surfaces with chemically defined patterns of alkyl- and aminosilanes. Proteins can be substituted for the alkylsilanes by adsorption onto lithographically defined surfaces. We have patterned several different proteins (collagen IV, fibronectin, laminin, and bovine serum albumin) and found their antibody and cell recognition properties remained after the lithographic and silyation steps. The spatial uniformity of patterned coverslips was assessed using an amine-specific fluorescent probe and immunochemical staining of proteins. Organosilane immobilization was confirmed with X-ray photoelectron spectroscopy (XPS) and contact angle measurements. Angle-resolved XPS measurements were also used to estimate the thickness and percent coverage of the organosilane overlayers.

We have analyzed attachment, guidance, and migration of mouse neuroblastoma cells on microfabricated surfaces [4, 5]. Interference reflection microscopy (IRM) was used to visualize sites of cellular attachment on substrates (focal and close contacts), and image processing techniques were used to quantify the results. Cells attached to and extended neurites on each of the substrates we tested. IRM images of growth cones displayed similar gray levels on amine, alkane, fibronectin and albumin substrates, whereas images on laminin and collagen were brighter. Brightness on laminin substrates was correlated with less area of focal contact and greater area with no contact. When cells were provided with choices on patterned substrates, they displayed the following preference of attachment: laminin, fibronectin, collagen > amine > alkane > albumin. This hierarchy reflected greater total surface area on preferred substrates. There was, however, no correlation between the hierarchy and the area corresponding to focal and close contacts or the percentage of contacts on different substrates. Our results support several recent observations which demonstrated that guidance was not correlated with differential adhesivity. These results are more in line with the theory that guidance is controlled by a signal transduction mechanism that couples locomotion with activation of membrane receptors.

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WEDNESDAY, MAY 11, 1994

SESSION 5
USE OF MICROFABRICATED DEVICES TO STUDY CELL FUNCTION AND DEVELOPMENT
Session Chair: Lynn Jelinski

"Micro-control of Neuronal Outgrowth"
Helen Buettner
Rutgers University, New Jersey

"Biofunctionalized Membranes on Solid Surfaces"
Robert Tampé and Erich Sackmann
Max-Planck-Institüt, Martinsried, and Technical Universität Munich, Germany

"Formation of a Simplified Brain on Microfabricated Electrode Arrays"
A. Kawana
NTT, Japan

"Microfabricated Surfaces in Signaling for Cell Differentiation in Fungi"
H.C. Hoch
Cornell University, Geneva, New York

Micro-control of Neuronal Outgrowth

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The ability to control outgrowth by nerve cells has important implications for improving nerve regeneration, investigating nerve development, understanding neural behavior, and incorporating aspects of neural structure and function into advancing medical and computer technologies. Ultimately, it is desirable to control growth at the single cell level and at micron dimensions, i.e., dimensions on the order of the cell features involved in the process. To achieve this goal requires a quantitative understanding of the fundamental mechanisms by which neurons develop and function, and how these mechanisms govern the response of the cell to specific environments.

Nerves develop at the cellular level by the outgrowth of long, slender processes from the cell body. These processes, the axons and dendrites (collectively referred to as neurites), eventually extend throughout the body, connecting neurons and innervation targets in a vast neural network that defines the basic structure of the nervous system. Neurite outgrowth is guided by a sensory motile apparatus at the neurite tip called the growth cone. A spread and flattened structure, the growth cone radiates fine spikes, or filopodia, around its lamellipodial periphery. The interaction of filopodia with elements of the extracellular environment is believed to play an important role in guiding neurite outgrowth and thus the development of neural architecture.

One key means by which filopodia may be involved in neurite outgrowth is by the remote sensing of discrete cues, often termed guideposts, in their environment. Both in vitro and in vivo studies have shown that filopodial contact with remote cues can initiate rapid neurite advance to the point of contact. Proper placement of such cues in series faithfully reproduces stereotyped pathways of extension during development. However when cues are small and sparsely located, as has typically been the case, contact is a probabilistic event raising questions as to how successful development is ensured and whether other more subtle guidance mechanisms are also required in concert.

To begin to address these questions quantitatively, we have developed a mathematical model of growth cone behavior in micropatterned environments, with emphasis on the response to guidepost cues. The model is based on parameters of growth cone movement and filopodial dynamics that we have measured experimentally, and it can be used to simulate neurite outgrowth in a variety of configurations. Comparison of model results with experimental data indicates that the model can reliably predict outgrowth in at least a simple in vitro patterned environment and can account for some in vivo observations that have been made to date. Thus the model serves as a conceptual framework for investigating cellular mechanisms in response to micro-heterogeneities and provides a basis for designing appropriate experimental systems, particularly microfabricated devices, for further study of this problem. The combination of precisely engineered, well-defined micro-featured environments and quantitative approaches to analyzing cell behavior in these environments offers tremendous potential for furthering our insight into the development and function of neurons as well a wide variety of other cell types whose behavior may depend on similar mechanisms.

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Biofunctionalized Membranes on Solid Surfaces

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In nature, cells sense their environment across membranes that display a vast variety of receptors and transport proteins. Because of their high adaptability, membranes have evolved to produce a wide functional spectrum. For the bioengineer, membranes offer an ideal tool to organize molecules in two dimensions. In engineering and physics, recent developments have yielded nano-techniques and surface-sensitive methods to design and investigate solid surfaces.

We are developing composite membrane systems on solid surfaces, such as SiO_2, Si, Au using vesicle fusion, Langmuir-Blodgett-techniques, self-organization, and newly synthesized functionalized lipids or polymers. In addition, we are interested in the two-dimensional pattern formation of membrane molecules by micro electrophoresis and phase segregation. We characterize biofunctionalized solid surfaces by electrical (e.g. impedance spectroscopy) and micro optical surface-sensitive methods, such as (e.g. micro fluorescence, surface plasmon spectroscopy ellipsometry, contrast interference microscopy).

In addition, we have introduced near infrared plasmon spectroscopy (NIR-SPS) as a tool to investigate the recognition of biomolecules on these biofunctionalized substrates. Working at a wavelength of 1300 nm, where both silicon and water exhibit a transmission window, sensitive detection of surface adsorption and binding is possible. The use of near IR wavelength light in a surface plasmon sensor not only provides higher surface sensitivity than working at visible light, it also permits the combination of NIR-SPS with electron or electro-optical devices on silicon substrates by nano-technology.

Formation of a Simplified Model Brain on Microfabricated Electrode Arrays

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The remarkable capacity of the brain for information processing remains poorly understood, because of the vast number of neurons and the overwhelming complexity of their interconnections. The main purpose of this study has been to understand the operation of the single neuron and the neural networks in the brain by investigating simpler, artificially cultured neurons and neural networks formed from biological neural cells.

Culture systems of vertebrate neurons provide a useful methodology for studying the physiology of neurons and neural networks in a simplified controlled environment. Recent progress in the technique of cell culture has made it possible to culture neurons from the brain and to form synaptic connections among them. However, neurons cultured in the usual way present a number of difficulties for studying the details of signal transmission and processing in neurites and networks. To achieve an understanding of signal transmission and processing, the morphology of the neurites should be simplified by controlling the outgrowth direction and developing technology to stimulate them at precisely localized points. For these purposes, we have developed a novel method of cell culture using substrates with patterned microgrooves and electrodes both of which were assembled using microfabrication technique.

The ability to stimulate and monitor the activity of neurons and neural circuits non-invasively is essential to understand their function. Planar electrode arrays were fabricated on silica glass by conventional photolithography. The spontaneous activity of neurons was monitored simultaneously through groups of electrodes. The period of bursting activity, which was synchronized among the electrodes, could be controlled by stimulation through these electrodes. This is very useful in probing the function of networks and to measure the effects of stimulation on the development of networks.

Clearly, it would be extremely useful if we could construct real neural networks in an analogous way to that in which integrated electronic circuits are built. We have established the potential for such a technique using substrates with wells and grooves and metal masks to control the position of neurons. We believe these methods should be powerful tools for understanding the operation of cultured neural networks, and ultimately of the brain.

Microfabricated Surfaces in Signaling for Cell Differentiation in Fungi

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Many fungi use cues inherent on the surfaces which they live for both growth direction and differentiation. Particularly unique are some leaf colonizing fungi that sense topographical features of the substratum to initiate pathogenic relationships with their hosts. One group of fungi, collectively known as the "rust fungi", cause serious losses in many agronomically important crops such as wheat, corn, and coffee. These rust fungi grow on leaf surfaces as thread-like hyphae toward stomata where they cease growth and develop appressoria, a specialized infection structure that constitutes an enlargement of the hypha tip facilitating entrance of the fungus into the leaf. The bean rust fungus, *Uromyces appendiculatus*, as well as nearly all other rust species, recognize stomata by the topographical characteristics of the surrounding guard cells. To understand better how these fungi perceive and mediate topographical signals, electron beam lithography was used to microfabricate specific topographies in silicon and quartz substrata on which the fungi were grown. Using polystyrene replicas made from topographied silicon wafer templates, we determined that the signal for development of appressoria was an abrupt change in elevation of the substratum and that this height (0.4-0.7 μm) corresponded very closely to the heights of the topographical features surrounding the stomatal guard cells. The fungal cells also respond to topographical features by growing perpendicular from them, a feature that has been exploited in microfabricating bull's-eye targets to guide cell growth to pre-determined locations. The behavior and function of fungal cells are becoming better understood through the use of these and other microfabricated surfaces and devices.

WEDNESDAY, MAY 11, 1994

SESSION 6
BIOLOGICAL PROCESSES AS TOOLS AND PROBLEMS APPLICABLE TO NANOFABRICATION
Session Chair: Harvey Hoch

"Nanoscale Biomechanics"
Robert Campbell
U.S. Army Research Office, Research Triangle Park, North Carolina

"Contemporary Problems in Biology: Contractile Materials"
G. H. Pollack
University of Washington, Seattle

"Force Generation by the Microtubule Based Motor Protein Kinesin"
F. Gittes and J. Howard
University of Washington, Seattle

"Microsensors and Microstructures for Biomedical Applications"
M. Esashi
Tohoku University, Japan

"Confocal Imaging of Flows in Microvessels"
Mary Lowe and Darren Hitt
Loyola College, Baltimore, Maryland

"Nanodesigning in Biological Composites"
Mehmet Sarikaya
University of Washington, Seattle

Contemporary Problems in Biology: Contractile Materials

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This presentation offers an overview of the field of contraction and motility, and the means by which nanofabrication might play a role in the future.

The mechanism of contraction is unsettled. A popular theory, which has been around for forty years, proposes that microscopic filaments slide past one another, propelled by oar-like action of proteins known as cross-bridges. Although this theory is widely invoked, there is mounting concern about the lack of agreement between prediction and observation. Attempts to confirm the oar-like action, for example, either through use of molecular probes bound to the cross-bridge, or through electron microscopy, have failed to detect the rotational motion predicted by the theory. Likewise, measurements of the consumption of chemical energy have led to conclusions about ATP consumption that many regard as unrealistic.

Many alternative models have been proposed over the years, but two are gaining increasing recognition. In one theory, it is a helix-coil transtion in a portion of the myosin (cross-bridge) molecule that brings about tension development, and drives contraction. In another, it is a "crawling" of one of the filaments (actin) over the other, that is the driving force. Both these alternative theories are gaining substantial experimental support, and are being looked at with increased attention.

Experimental approaches to the exploration of the molecular mechanism of contraction involve progressively smaller preparations. Two of these will be discussed. In one, known as the in vitro motility assay, molecules of myosin are laid out on a flat substrate, and fluorophore-labelled actin filaments are added. In the presence of ATP, the actin filaments translate over the field, and can be observed by fluorescence microscopy. In order to explore the transduction mechanism, several groups are attempting to measure the level of force on the actin filament. One convenient method of doing this is to employ a nanofabricated beam, whose deflection is proportional to force. Nanofabrication can also be used to manufacture "tracks" along which myosin molecules can be laid.

A second preparation that has come into existence is the single myofibril. The single myofibril is somewhat larger in scale than the in vitro motility assay (1 um diameter), but retains the filament-lattice structure characteristic of the intact specimen. It is in fact the smallest preparation that retains this feature. Measurements of tension and length of individual sarcomeric units can be made with precision. Again, nanofabricated devices can be used either to measure tension, or as motors to impose programmed length changes on the myofibril.

The use of these two experimental preparations will be discussed, along with some implications as to which of the above-mentioned models are favored by the results.

Force Generation by the Microtubule-based Motor Protein Kinesin

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Kinesin is a force-generating enzyme, or motor protein, which converts the free energy of the gamma phosphate bond of ATP into mechanical work. This work is used to power the transport of intracellular organelles along microtubules, cytoskeletal polymers of the heterodimeric protein tubulin. We are attempting to understand the workings of this tiny motor: how much force can it generate, how far does it move for each ATP hydrolyzed, and how efficient is the engine?

To answer these questions we have developed cell-free assays in which the movement of individual microtubules across a kinesin-coated glass surface is directly observed under the microscope. By decreasing the density of kinesin on the surface we have shown that a single kinesin molecule suffices to move a microtubule (1). Using this single-motor assay, we have measured the single-molecule force using two different approaches. In the first, the load on the motor is increased by raising the viscosity of the solution through which the microtubule moves 100-fold; the decreased speed of movement indicates that a single motor can work against a load as high as 4-5 pN (2). In a second approach, we have used the microtubules themselves as calibrated force transucers of known bending stiffness (3): observations of the buckling of these microtubules indicate that kinesin can work effectively against elastic loads of up to 4 pN (4). This single-motor force probably does not depend strongly on the orientation of the microtubule relative to the motor's attachment to a glass or organelle surface because of kinesin's extremely high torsional flexibility (5).

We have elucidated the path which kinesin follows along the surface of a microtubule. Microtubules polymerized *in vitro* with 12, 13, or 14 protofilaments respectively rotate clockwise, not at all, and counterclockwise when moving across a kinesin-coated surface (6). The pitch and handedness of the rotation matches the "supertwist", measured by electron cryo-microscopy, of the protofilaments about the microtubule's long axis. This proves that the motor follows the protofilament axis, and, since there is only one high-affinity kinesin binding site per tubulin dimer, the distance moved per ATP must be an integral multiple of 8 nm, the spacing of the dimers along the protofilament. Combining this distance with the above force measurement indicates that the thermodynamic efficiency of the motor exceeds 40%.

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Microsensors and Microactuators for Biomedical Applications

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Active and multifunctional catheters for use in instrumentation and treatment inside a blood vessel are under development. It will have to be equipped with more than one sensor and with actuators to allow bending. Communication and control technologies will also be required to minimize the number of lead wires. For this purpose, a common two lead wire system in which the two lines supplying power also convey signals and select the sensor or the actuator to be activated; micropackaging technology for assembling and microactuator techniques are also being studied. Silicon micromachining based on integrated circuit technology and three-dimensional microfabrication technologies is applied for the catheter.

Packaged and integrated microsensors
A packaging technology which will integrate a capacitance detection circuit, a capacitive sensor in a parallel electrode structure, an electrostatic actuator and a resonator is developed. This technology makes small, cheap and reliable sensors available. Fig. 1 shows an integrated capacitive-accelerometer which employs an electrostatic force-balancing servomechanism. An integrated capacitive pressure sensor and a three-axis force-balancing accelerometer were also developed with this packaging technology. Silicon micro resonators can have very high quality factor because of its small internal friction, which enables highly sensitive sensors. Resonant infrared sensor of which resonant frequency is modulated by radiation-induced thermal strain variation, and resonant angular rate sensor using electro-magnetic excitation and capacitive detection were developed.

Microactuators and three-dimensional microfabrication
A distributed electrostatic micro actuator (DEMA) is proposed. The DEMA has many serially connected driving units which consist of two wave-like electrodes facing each other. Large electrostatic force and large displacement can be obtained by narrowing the gap and by serially connecting the driving units. The structure fabricated by photolithography and cupperelectroplating is shown in Fig. 2. Displacement obtained was 28 μm (about 10%) when applied voltage was 160 V. High aspect ratio microstructure of polyimide was made by reactive ion etching using oxygen gas. The width and the depth were 5 μm and 75 μm respectively. Eximer laser assisted Cr CVD on non-planer polyurethane catheter are developed for tactile sensor at the tip.

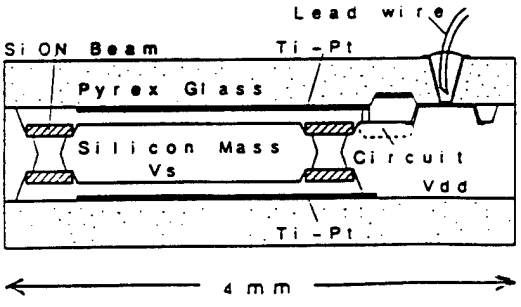

Fig. 1. Integrated capacitive accelerometer

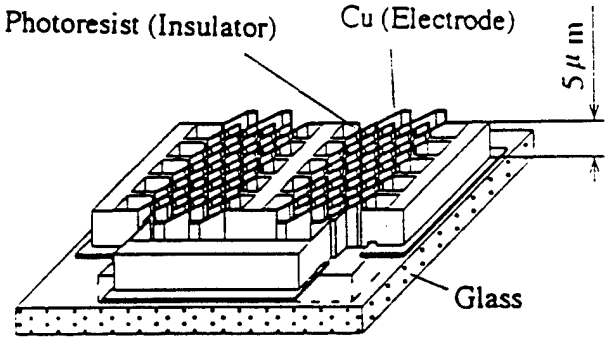

Fig. 2. Distributed electrostatic microactuator

Confocal Imaging of Flows in Microvessels

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and

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Venular flows may be characterized by two converging streams in vessels 20-200 um in diameter. In a simplified model of blood flow in venules, finite element computations reveal the shape of the interface between two Newtonian fluids converging at a T-bifurcation. We are currently studying this phenomenon experimentally by fabricating artificial networks of microchannels in glass with physiologically realistic dimensions. Using confocal microscopy, we have made the first observation of the interface in three dimensions.

Our samples are obtained from MMR Technologies Inc. The fabrication method involves photolithographic patterning of glass substrates followed by abrasive etching with a fine aluminum oxide powder. A flat glass cover slide is then bonded to the etched glass slide by means of a low melting point glass. This technique results in channels which possess a D-shaped cross section and a surface with roughness on the scale of 1.4 um. To make tubing connections, brass hose barbs are glued onto the sample.

By injecting fluorescent aqueous fluids in the channels, we can study the interface in three dimensions using a laser-scanning confocal microscope (Bio-Rad MRC-600), and volume rendering software. We have also obtained time series measurements of blood flow in tubes using this apparatus. Additional experiments with blood are underway. This poster will discuss the microfabrication method, confocal imaging, experimental data on blood flow, and a comparison with computational results.

*Supported by NSF Award CTS-9253633

Nanodesigning in Biological Composites

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Biological hard tissues, such as bone, dentin, and mollusk shells, are composite materials incorporating both inorganic phases (such as carbonates, phosphates, and oxides) and inorganics (macromolecular structural units including proteins and polysaccharides). These materials have unprecedented physical properties that are often multifunctional due to their hierarchically ordered structures through the dimensional spectrum from macro- to nano-meter and molecular scales. Biological materials are a source of inspiration for design and processing of technological materials, *biomimetics*, based either on their structures and functions, *biomimicking*, or synthesis and processing, *bioduplicaton.*

In this presentation, we plan to discuss our current work on these unique biological composites: (i) nacre section of the mollusk shells, where the structure consists of submicroscale laminates of $CaCO_3$ platelets separated by 10 nm organic matrix; (ii) antler bone, where highly organized ultrafine disk-shaped apatite crystals form in a collageneous matrix; and (iii) ultrafine particles in magnetotactic bacteria. In each case, current understanding of the critical issues, such as formation and growth mechanisms, organic matrix-inorganic phase structural relationships, nature of the organic matrices, physical properties of the biocomposite and morphogenesis will be discussed in the context of biomimetic applications.

THURSDAY, MAY 12, 1994

SESSION 7
APPLICATIONS OF MICROFABRICATION IN MOLECULAR BIOLOGY
Session Chair: Harvey Hoch

"Technology Needs for the Human Genome Project"
David Burke
University of Michigan, Ann Arbor

"Use of Micromachined Structures for the Manipulation of Biological Objects"
Masao Washizu
Sekei University, Tokyo, Japan

"BIAcore: A Surface Plasmon Biosensor for Characterization of Biospecific Interactions"
M. Malmqvist
Pharmacia, Uppsala, Sweden

"Nanoscale Structures Engineered by Molecular Self-Assembly of Functionalized Monolayers"
D. Allara
Pennsylvania State University, State College

Technology Needs for the Human Genome Project

David T. Burke
Department of Human Genetics
Assistant Director for Technology Development
Human Genome Center
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The Human Genome Project is a large, directed effort to establish a data infrastructure for human genetics and the biological sciences. The scope and time-scale of the project were proposed in a 1988 National Research Council report *Mapping and Sequencing the Human Genome*. The 15-year project is jointly administered by the National Institutes of Health and the Department of Energy, and has a current-year budget of $170 million. The project has several goals, both explicit and implicit in the NRC report. The explicit goals are the determination of the complete DNA sequence of the human genome and the genomes of several experimental organisms. Complete sequencing implies the isolation of fragments of each genome as *clones*, the ordering of the cloned DNAs as a linear *genome map*, and the annotation of the map with biological information, such as the location of individual *genes*. The sum of this information is expected to provide a foundation for experiments in medicine and basic biology.

The NRC report anticipated three five-year periods, each defined by milestones unattainable by the then-available level of technology. To date, those expectations have been fulfilled, with technical developments occurring at a rapid pace. Most of the developments have been at the level of basic biochemical manipulation of DNA; including new methods for DNA isolation, large DNA fragment cloning, DNA amplification (PCR), and DNA chemical synthesis. These have led directly to a complete moderate-resolution genetic map and the first low-resolution clone map of the human genome. Detailed descriptions of several *experimental organisms* have also been completed. The second and third five-year periods are expected to refine the crude maps, annotate them with important biological information, and finally, complete the full DNA sequence.

The 10- and 15-year NRC goals are clearly not attainable with the current level of technology. Technical advances that increase sequencing abilities by over 100-fold must occur, not only to acquire the initial information but to implement the sequence information in solving real-world biological problems. An example is DNA-based disease diagnostics. It is not sufficient to simply discover the DNA sequence of a gene involved in a disease; that sequence must also be re-identified in thousands of individuals being tested for the disease. Although the Genome Project defines the three billion basepairs of the human genome as its goal, the demand for additional DNA sequence information resulting from that knowledge is unlimited.

Future progress in genome analysis will require improvements in all aspects of DNA biochemistry, sample handling, and data acquisition. Significant opportunities exist for technologies that have been established outside biology. Micro-scale technologies, specifically those using silicon-based fabrication, hold great potential. It is possible that all aspects of DNA isolation, purification, and sequencing biochemistry can be placed within a single, integrated platform. Ideally, data detection, analysis, and decision-making would also reside in the same system. Microscale biochemistry will reduce costs associated with expensive biological reagents, while lithographic fabrication techniques would provide inexpensive identical components. Finally, the integration of information-processing and sample-processing on a single platform holds the possibility of reducing human interaction in the *acquisiton* of data, while retaining interaction for its understanding.

BIAcore: A Sufrace Plasmon Resonance Biosensor for Characterization of Biospecific Interactions

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Interactions between biomolecules are the basis for life and in most cases a protein is one of the components. For a more detailed understanding of structure - function relationships the characteristic properties of the interaction must be known. Accurate measurements of physical-chemical parameters such as rate constants and affinity are important as well as determination of binding sites on the two molecules. These parameters are most important to measure when antibodies and other binding substances easily will be created by phage display of gene libraries[1].

A system for label-free analysis of biospecific interactions has been developed. The basic idea is to measure the biospecific adsorption of one component of a biospecific pair to the immobillized partner directly on the same spot and at the same time it occurs. The surface sensitive optical technique[2], surface plasmon resonance, SPR, has been used in combination with a carboxydextran modified gold surface[3]. A liquid handling cartridge[4] with flow channels and valves guides reagents and samples to the interaction position. The system has been used for rapid generation of kinetic and affinity information[5] of protein-protein, DNA-protein, DNA-DNA and receptor-ligand interactions. Label-free detection of interactions is general and applicable to any ligand - analyte pair. Kinetic information can be generated without purification of the components. With SPR the refractive index is measured on and in close vicinity of the surface that favours the analysis of large molecules. However, by competition analysis affinity and kinetic information can be generated also for small molecules. By using a dextran coupled to the gold surface a 100 nm thick hydrophilic micro matrix is created which can be used for analysis of the formation of multimolecular protein complexes.

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Use of Micromachined Structures for the Manipulation of Biological Objects

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Manipulation of biological materials, such as biological cells, organelles, molecules and membranes, constitutes an important unit-operation in biotechnology. Because the dimensions of these objects range from hundred of microns for cells down to nanometers for molecules, and they are often soft and fragile, their manipulation, especially on one-by-one basis, requires tools with comparable dimensions and special methods of actuation. For this purpose, micro-fabrication techniques based on photo-lithographic processes, which are used for the production of semi-conductor integrated circuits, provide ideal means to manufacture microstructured tools, while electrostatic effects, such as dielectrophoresis (DEP), electrostatic orientation and electrorotation, are well-suited for gentle actuation of these objects. In addition, because a microstructured electrode system has large surface-to-volume ratio, it allows the use of very high intensity fields without excessive temperature rise.

Cell handling devices combining micro-fabrication and electrostatic methods have been developed, and are named Fluid Integrated Circuits (FIC) because all components for cell handling, connected by fluid passages, are integrated on one substrate. Transport, sorting, and fusion of cells are possible with the use of well-defined field pattern in microfabricated electrodes and structures. The torque-speed characteristics of bacterial flagellar motors has been measured with the use of electrorotation in a rotating electrostatic field created by a multi-phase micro electrode system.

Molecular manipulation is another important field of the application of FIC. The electrostatic orientation effect makes it possible to stretch a DNA, which takes randomly coiled conformation in water due to thermal agitation, to its full length. The stretched DNA is driven by dielectrophoresis until one of its molecular end is in touch with the electrode edge. DNA molecular size can be determined by measuring the stretched length. Methods has been developed to immobilize the stretched DNA onto a substrate. By anchoring DNA at its molecular ends only, and keeping other part without contact to the substrate, sliding of RNA polymerase along DNA has been visualized. It has also been demonstrated that dielectrophoresis is effective for even smaller molecules such as protein. Its possible application is the separation of protein according to electrical polarizability.

Using micromachined structures, novel methods has been developed in such areas as handling and unfolding of chromosomes, fabrication of phospho-lipid black-membranes and liposomes.

It is hoped that the manipulation methods of these micrometer-sized objects, made possible with the emerging microfabrication techniques, will introduce 'spatial resolution' to the biology and biochemistry in which objects has conventionally been treated as suspension or solution in a tube.

Nanoscale Structures Engineered by Molecular Self-Assembly of Functionalized Monolayers

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Over the past decade there has been intensive activity in developing methods to assemble molecular-scale thickness coatings by spontaneous assembly from solution. Highly stable, densely-packed films can be prepared on varied types of substrates by either selecting specific chemistry to form molecule-substrate bonds or by inducing chemical "crosslinking" between the adsorbed molecules. However, few molecule-substrate combinations have been developed and the majority of studies and applications have centered on the specific combinations of organothiols on gold surfaces and organosiloxanes on silica surfaces. Thus one of the challenges ahead is to develop new combinations, guided by potential applications, e.g., semiconductor devices. In this regard, much of our recent work has been involved in developing methods of self-assembly of monolayers on substrates such as GaAs, TiO2 and bare Si for applications to the formation of ultrahigh resolution patterns down to the tens of nanometers scale. This work has been carried out in collaboration with H.G. Craighead and his co-workers at Cornell University and it has been shown that the combination of electron-beam irradiation followed by etchant solution exposure leads to the preferential etching of features in the irradiated areas. The promise of these applications is that the ultimate resolution of the surface features may be that of the molecular size, ~0.5 nm. Another important aspect of self-assembled monolayers is the ability to form these films from highly functionalized molecules thereby forming correspondingly chemically functionalized surfaces. The premier examples of this capability has been with the system of organothiols on gold surfaces. It is now well-known that by selection of functional groups with different donor-acceptor properties than that of divalent sulfur, S-Au bonds preferentially can be made to form leaving the other groups free to modify the ambient surface chemistry for applications involving such attributes as highly specific electrochemical, wetting and biological responses. Two challenges lie ahead here. One is to learn how to control spatial patterns of mixed functional groups on the nanometer scale, a challenge of particular interest in biological applications. In this aspect we have been actively working on both characterization and preparation methods. In a recent collaboration with P.S. Weiss and his co-workers at Pennsylvania State University, scanning tunneling microscopy has shown that nano-scale patterns can be formed by spontaneous phase segregation in monolayer films of very similar organothiol monolayers on gold surfaces. The other challenge is to extend the highly flexible multifunctional chemistry on gold surfaces to other substrates. In this regard we have had intial success in self-assembling functionalized monolayers on GaAs(100) surfaces. These types of structures offer opportunities to combine the electrical responses of the semiconductor with the potential chemical and biological specificity of the functionalized surface. Ultimately, the combination of natural and lithographic patterning will lead to a high degree of control over the chemical architecture of surfaces.

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Fabrication of Biomolecule-carrying Submicron Structures by Deep-UV and Electron Beam

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University of Oregon

The Creation of Biomolecular Arrays by Electrostatic Immobilization on an Insulator Surface Exposed to an Electron Beam
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Applications of Micro- and Nanolithography to Biological Problems

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Micro and nanolithography has recently captured the attention of both the biophysical and biological sciences communities because this technology allows one to build structures on the same size scale as biological organisms and molecules, and to mimic the structures that biological organisms exist in. Further, the extraordinarily small volumes and quasi-2-dimensional nature of microlithographic structures allows the scientist to study biological organisms at the {\em individual unit} level, in recognition of the extreme diversity that biological systems possess.

I will discuss 3 areas where my colleagues and I have exploited these frontier aspects of the technology: to create synthetic lattices to fractionate DNA molecules at the chromosomal length scale, to study the flow and response of red blood cells through a mimic of the arterial capillary bed, and to attempt to sequence chromosomal length DNA via a combination of attachment of DNA to nanoscale gold wires and STM spectroscopy.

Using Picoliter Reaction Vials

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Capillary electrophoresis (CE) is a technique which allows femtoliter volumes to be injected and separated. The low volumes used in this technique make it ideal for use in microenvironments, such as biological systems. The complex biological processes occuring in a system can often be determined only by looking at individual cells in a heterogeneous environment. Hence the use of CE for single cell analysis. In order to isolate a single cell, a very small reaction vial is necessary. Vials with sizes of 10, 20, 50, and 100 pL have been fabricated with the assistance of the National Nanofabrication Facility.

CE with electrochemical detection is used with the vials to monitor release from single bovine adrenal cells. These cells release catecholamines which are easily oxidizable. There are other analytes present, however, which cannot be detected by this method. CE with fluoresence detection is used to analyze the components of whole PC12 cells. Derivitazation is necessary for single cell analysis in CE because many analytes are neither natively fluorescence or electrochemically active. By using the microvials for this derivatization step, dilution of the sample is reduced, and the entire cell contents can be analyzed at once.

Red Blood Cell Dynamics in a Micro-fabricated Capillary Bed.

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We have used microlithographic techniques at the National Nanofabrication Facility (NNF) to fabricate an environment designed to simulate the capillary bed: a 4 micron high obstacle array consisting of narrow 4 micron channels etched into silicon. Since the human red blood cell is about 7 micron in diameter, these channels force the cells to deform in a way similar to the way they pass through capillaries. The array is sealed with a Pyrex lid, and we observe cells passing through the channels using epi-brightfield and epi-fluorescence video microscopy. A major feature of this technology is our ability to monitor hundreds of cells simultaneously as they are forced through the array by a hydrodynamic flow. We have found several remarkable and unexpected phenomena of human red blood cells as they pass through the array: (1) There is a large dispersion in cell velocities (three fold) in spite of the narrow range of red blood cell diameters. (2) There is no correlation between cell diameter and speed in the array. (3) Cells can become spontaneously very rigid on the sub-second time scale, can back out of narrow openings against the flow, and can become flexible again on the sub-second time scale. We will show video highlights of our findings.

Development of an Array-Based Multi-Analyte Immunosensor

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The fabrication of microsensor devices capable of performing simultaneous detection of multiple analytes is currently of interest in clinical, forensic, and environmental laboratories. A multi-analyte sensor would make possible rapid prescreening of complex samples with minimum sample pretreatment, operator input, or turnaround time.

Our own effort to develop this type of sensor is based on the use of multiple monoclonal antibodies to serve as molecular recognition sites for specific analytes (antigens) of interest. By immobilizing these antibodies onto a solid support in discrete locations, a controlled and individually addressable array could be produced. Analyte identification would then be accomplished by determining the location on the substrate from which the signal was produced. Fluorescence detection would offer the required sensitivity and selectivity to probe binding at the patterned surface. Signal generation could be achieved by performing a competitive immunoassay using fluorescently-labeled analogs of the analyte of interest. If the sample contained any of the molecules with which the antibodies show specific binding, discrete regions of the support would show decreased fluorescence. This would obviously occur because analytes present in the sample could effectively compete with the fluorescent analogs for binding at specific sites on the support. The resulting decrease in fluorescence could be easily calibrated to reflect the target analyte concentration present in the sample. A fiber optic bundle or confocal fluorescence microscope could be used to investigate and address individual regions of the substrate, coupling signal transduction and analyte identification.

The main challenge faced when attempting to produce this type of biosensor is how to overcome the myriad of problems encountered during sequential antibody patterning. My presentation will outline several possible methodologies that can be used to best address the following questions crucial to the production of a microfabricated, patterned, immunoassay-based biosensor:

1) How does one minimize the deleterious effects non-specific adsorption of antibodies to the substrate and/or to other antibodies?
2) What is the ideal solid support on which to pattern antibodies for this particular application?
3) How can patterns of sufficient resolution be most efficiently produced without employing denaturing conditions?
4) What are the factors which set the practical upper limit on the number of immobilization iterations?
5) Are there advantages associated with patterning antigens, as opposed to antibodies themselves?

Synchrotron Radiation Spectromicroscopy: a New Fundamental Tool in Biology

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In the past twenty years absorption spectroscopies has been a leading tool in materials science, but, due to the lack of lateral resolution, their impact in the life sciences has been minimal [1]. Recent instrumentation improvements make it possible to perform absorption *spectromicroscopy* on the submicron scale, paving the way for experiments on biological interest [2]. The X-ray Secondary electron Emission Microscopy (XSEM) version of synchrotron radiation spectromicroscopy is able to produce images of microscopic areas of biological specimens, distribution maps of specific elements, and absorption spectra from micro-regions of relevance, such as specific cell structures recognizable from their morphology in the images. From these spectra it is also possible to retrieve information on the chemical status of each element in the sample. We present a short review of the results produced by XSEM spectromicroscopy [3-5]. Among these results, we note the localization of toxic and trace elements on microscopic areas of neuron networks in culture. The most interesting result was obtained on aluminum, the element known to be involved in human pathologies like Alzheimer's disease, Parkinson Dementia and Guam [6]. In cerebellar granule cells' cultures, we observed with XSEM, aluminum accumulated selectively in Purkije neurons, with very good statistical significance [5]. Naturally present elements, as well as the neurotoxic zinc, cobalt, iron and chromium have also been investigated, and the corresponding results are presented.

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Adhesion Forces Between Individual Ligand - Receptor Pairs

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The adhesion force between the tip of an atomic force microscope cantilever, derivatized with avidin, and agarose beads with either biotin or desthiobiotin was measured. Under conditions where we allowed only a limited number of molecular pairs to interact, we found the force required to separate tip and bead to be quantized in integer multiples of 170± 10 pN for biotin and 125± 20 pN for desthiobiotin. We conclude that the measured force quanta are the unbinding forces for individual molecular pairs.

Investigation of Microstructure, Crystallography and Mechanisms of Mineralization Regulated by Organic Molecules in Biogenic Calcium Carbonates from Mollusk Shells

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Ultrafine composite materials consisting of calcium carbonate and organic matrix phases are grown by several species of marine organisms and exhibit characteristics markedly different than those of geologic calcium carbonates of the same polymorphic form. It is thought that the mechanism for formation of these composites involves interaction of the organic component provided by the organism and its effect on crystallization from solution of calcium carbonates. In this poster session the work presented involves microscopic analysis of a specific example of biogenically formed composite, nacre from several different molluscan shells. For samples of this material, analysis of crystallographic orientation of individual platelets of aragonite within the composite brick-and-mortar like nacre is described. Characteristics of this biogenic aragonite which distinguish it from geologic aragonite are presented. Morphological and crystallographic features, defects, and other specific aspects which distinguish biogenic and geologic aragonite are shown. Preliminary results of investigation of regulation of mineral formation by organic matrix components is presented for the formation of nano-layered materials based on biomimetics principles.

Preparation and Characterization of Nanofabricated Fibers for use in Model Studies of the Mechanisms of Lung Fibrosis

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Inhaled particulates in the lung induce the phagocytic activity of alveolar macrophages. Activated macrophages produce many chemical substances and mediators, some of which are believed to initiate a chain of events which lead to the proliferation of connective tissue, the formation of fibrotic and granulomatous lesions, and lung cancer. However, these lung diseases are only observed for particulates of certain materials and certain size ranges. For example, silicon oxide particles and asbestos fibers can be carcinogenic, while titanium oxide particles are observed to be "inert". The response is also dependent upon the specific form of asbestos or silicon oxide. Although these phenomena have frequently been observed in animal studies as well as human patients, the mechanisms by which alveolar macrophages are stimulated by inhaled particulates to promote fibrogenesis are still not understood.

One problem in studying inhalable particles and fibers of micron dimension is the difficulty to control their surface properties. Additionally, commercially prepared particles and fibers have inherent size distributions within a given sample, and chemical composition can vary even within a particular production lot. Using nanofabrication techniques, we have been able to produce fibers of controlled compositions and tight dimensional distributions. These fibers have been made in Au, Ti (oxide) and Si (oxide), in both amorphous and crystalline forms, having dimensions 0.1 μm, 1 μm, and 10 μm. Another benefit of nanofabrication is the ability to produce fibers which have the same composition and structure as flat surfaces, as well as surfaces with patterned topographies. These nanofabricated fibers and surfaces will be used in a study which investigates the roles of surface chemistry, fiber dimension and crystallinity in the phagocytic response of alveolar macrophages in cell culture. By preparing the same surfaces and fibers in different materials, we are able to test for synergistic effects between dimensional aspects and surface chemistry on cellular behavior. The fabrication techniques and characteriza-tion of these fibers will be presented. Reference will be made to ongoing biological studies at the Dept. of Environmental Medicine, Univ. of Gothenburg, Sweden.

Nanostructured Semiconductors for Biophotonics*

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Biophotonics is the science and technology of light quanta (photons) and their interaction with biological matter. Artificially structured semiconductors can play an important role in biophotonics because of their remarkable ability to generate, control, transmit, and detect light quanta. Indeed, modern growth techniques like molecular beam epitaxy produce semiconductor crystals layered with nanometer precision whose photonic properties can be programmed by computer control. Further processing by electron-beam lithography and ion beam etching provides precisely defined artificial structure across the wafer surface. The synthesis of layered epitaxy and surface processing yields 3-dimensional architectures with unprecedented photonic properties.

Recently, we have invented a *biocavity laser* which provides high contrast, coherent light images and spectra of the DNA/protein complexes in living human cells. The biocavity laser is a synthesis of nanolayered semiconductor technology, ultrasensitive laser microscopy and spectroscopy, and biological microstructures to non-invasively probe individual cells. Laser action in microcavities formed with human red and white blood cells has been observed. Both cell types serve as optical waveguides to confine laser light in a Fabry-Perot resonator formed with a semiconductor wafer. Extremely high contrast microscopic images of the cell and its internal parts are observed in the near infrared at 820 nm. These images correspond to electromagnetic modes of cell structures and are sensitive to the distribution of DNA/protein complexes within the cell. The images provide coherent light maps of the cell membrane, nucleus and other intercellular bodies. When light from these images is resolved with a spectrometer, the measured spectra exhibit sharp peaks associated with individual optical modes defined by the cell structures. These peaks provide quantitative information about DNA/protein concentration in cells. This ultra sensitive biocavity laser represents a new technique for studying biological material. It may be useful for probing the human immune system, detecting genetic defects at the DNA level, and sorting cells non-invasively.

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Nanostructures for Bioelectronics and their Applications in Cell Signalling

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Bioelectronics is concerned with the study of the interface between biological and electronic systems. The dimensions of biological molecules indicate that nanofabrication technology is particularly well suited to the development of new techniques for research in this field. In this paper we describe the fabrication of a novel geometry of ultra-microbiosensors for studying the production of compounds produced by individual cells. The electrode structure consists of gold-band electrodes, fabricated on glass and insulated using silicon nitride. Individual electrodes, which are 200 nm wide, have been modified using suitable enzymes and proteins and initial low current measurements (~pA) have been made. The compounds that we are primarily interested in detecting include glutamate (1) and superoxide anions (2).

The problem of positioning a single cell within this sensing geometry is addressed with dielectrophoresis. This technique uses non-linear electric fields, maintained between lithographically defined electrodes, to manipulate individual cells into precise positions. A related technique is also described in which we can differentiate between live and dead cells, and between cell types.

The fabrication facilities within the Department of Electronics and Electrical Engineering that have been used in this study include a Leica-Cambridge EBPG5-HR lithography system offering a 12 nm spot and 50 nm positioning accuracy over 125 mm and a modified JEOL 100CX-II TEM offering a 3 nm spot and a demonstrated resolution below 10 nm in lift-off. The lithography systems are supported by a large range of micro/nano electronic processing facilities including oxide and nitride deposition, metal deposition, wet and dry etching as well as STM, AFM and high resolution SEM for the characterisation of structures.

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Use of Microfabricated Devices in Understanding the Effect of Surface Charge Heterogeneity on Biological Response to Implants

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The long term success of an implant, whether it be a hip-replacement, or a pacemaker lead is determined by the initial response of the host cells to the surface chemistry, morphology, and electrical properties of the implanted material. Much of the developmental research in biomaterials has ignored the influence of the latter surface feature. The recognition of the influence of surface charge density and polarity of homogeneously charged materials on the biological activity of fibroblasts[1] has prompted further investigation into the surface electrical variables influencing other host cells. Since surfaces of implant materials are inhomogeneous, it is our goal to investigate the effect of electrical surface heterogeneity of polymeric biomaterials on the response of mammalian cells, while controlling surface chemistry and morphology.

The key to this goal is to fabricate surfaces that can bear a controlled heterogeneous charge distribution. In collaboration with the National Nanofabrication Facility at Cornell, we have designed and fabricated an array (8 mm x 8 mm) of 3.3 μm wide linear electrodes separated by 0.7 μm insulators. A 900 Å layer of SiO_2 was deposited on the electrodes followed by spin coating with a thin layer of polystyrene. By applying the appropriate voltages (alternate positive and negative of equal magnitude) on the underlying electrodes, a corresponding mirror charge can be induced in the sub-micron thick polymeric film which produces a net neutral surface at a macroscopic level and a heterogeneously charged surface at the microscopic (cellular) level. The predicted electrical field from the induced charge was modeled numerically.

The fabricated surfaces were seeded with human white blood cells (peripheral blood monocytes) in the presence of growth medium. The cells were allowed to incubate on the polymeric surfaces with 4 different levels of induced charge (treatment) or without induced charge (control) for various lengths of time and were then challenged with phagocytic particles (trigger).

Cell spreading is a function of heterogeneity of surface charge and surface charge density in this model. Cells spread more on the heterogeneously charged surfaces (as evidenced by increased surface area and perimeter and decreased roundness) as compared to the control. There are also quantitative differences in the cytokines present in the supernatents among the cultures from the charged and control surfaces.

Surface electrical heterogeneity influences the human monocytes in this model. The nanofabrication technology may provide a new dimension in the design of novel biomaterials with controlled static charge properties to obtain a more favorable response from local host cells.

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This research has been funded by Food and Drug Administration, Rockville, MD, USA and the Hunter Endowment of Clemson University, Clemson, SC, USA.

Patterned Culture Surfaces for Studies of Neuronal Adhesion and Guidance

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Culture surfaces patterned with spatially distinct chemistries can be used to study the molecular interactions underlying cell attachment, guidance, and migration [1]. Patterned surfaces also allow the formation and study of neural networks *in vitro* by segregating cell types and guiding cell appendages to locations where specific intercellular connections can occur [2]. Patterned surfaces may also be helpful for guiding nerve regeneration *in vivo* [3].

We have used microfabrication techniques to pattern culture surfaces with distinct chemistries [4-6]. By combining silane-coupling chemistry and photolithography different silyated molecules are covalently attached and patterned on silicon-based substrates. Recently we modified this method to be faster, less expensive, and more feasible in a standard biological laboratory [5]. Briefly, glass coverslips are spin-coated with photoresist and exposed to UV light through a mask. After removing exposed photoresist, alkylsilanes (dimethylsilane, DMS; hexadecylsilane, HDS; or octadecyldimethylsilane, ODDMS) are bound to the exposed regions. The remaining pattern of photoresist is then removed, and aminosilanes (ethylenediamine-propylsilane, EDS) are bound to the newly exposed glass regions. This process yields surfaces with chemically defined patterns of alkyl- and aminosilanes. Proteins can also be substituted for the alkylsilanes by adsorption onto lithographically defined surfaces. We have patterned several proteins (collagen IV, fibronectin, laminin, and bovine serum albumin) and found their antibody and cell recognition properties remained after the lithographic and silyation steps.

The spatial uniformity of patterned coverslips was assessed using an amine-specific fluorescent probe and immunochemical staining. Organosilane immobilization was confirmed with X-ray photoelectron spectroscopy (XPS) and contact angle measurements [5,6]. Angle-resolved XPS measurements were also used to estimate the thickness and percent coverage of the organosilane overlayers.

Cell attachment, guidance, and migration on patterned surfaces was also analyzed [1,5,6]. Mouse neuroblastoma cells were cultured on patterns of amines, alkanes, and proteins in the following combinations: alkane-glass, protein-glass, alkane-alkane, amine-alkane, amine-protein. Observations of cell distribution 24 hours after plating revealed the following hierarchy of preferences: collagen IV, fibronectin, laminin > EDS, glass > DMS, ODDMS, HDS, BSA. Avoidance of BSA indicated that the cellular preferences for extracellular matrix proteins were due to specific properties of these proteins. The preference for matrix proteins over EDS indicated that this specificity was not simply due to positively-charged moieties of the matrix proteins. Interference reflection microscopy was used to observe the attachment sites of cells on patterned surfaces. On amine-alkane surfaces, cells and growth cones displayed similar adhesion profiles on both regions, even though cells were strongly guided by the amine paths. On laminin-EDS surfaces, cells were less adherent on laminin regions though they were guided by these regions. This suggested that cells can be guided by mechanisms other than the most adhesive surfaces.

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Spatially Constrained Electrodeposition

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A new microfabrication technology capable of producing truly three dimensional metal structures by rapid localized electrodeposition is introduced. The technique is being developed in response to a need for sub-micrometer resolution micro-fabrication methods in our laboratory to assist in the construction of micro-manipulators and micro-robots. For example, a micro-end-effector is required to complete a tele-operated micro-surgical robot, being developed to perform corneal and retinal surgery. In another project in which we have built an 8-limbed nano-motion robot a multi-degree of freedom micro-hand is needed to manipulate muscle cells and other single cells for mechanical and optical probing.

Localization of electric field and thus of the electrodeposition is achieved by placing a pointed electrode near the deposition surface, thus making use of the field concentration at the tip. Finite element analysis was used to predict deposition profile. The analysis suggests that the profile is approximately Gaussian in shape, with a full width half maximum (FWHM) of less than twice the tip diameter being achievable given a tip to surface distance of about one diameter. By moving the tip appropriately with respect to the deposition surface three dimensional objects can be produced. Note that the deposition nature allows truly three dimensional structures to be created, unlike photo-lithography based techniques, and has the potential to produce closed interstices-impossible using machining methods.

The technique has been implemented using platinum wires with tips etched to about 25 mm in diameter. Feature sizes down to 30mm have been achieved, including cylinders more than 1500 mm high. Formation rates per unit area of greater than 6mm/s were achieved, an order of magnitude greater than rates observed in conventional electroplating. Given that diffusion is the major rate limiting factor in electrodeposition, geometrical considerations imply that formation density will be inversely proportional to tip diameter, thus leading to increasing rates as tip size is reduced. Using finer tips such as those commonly employed in STM, we expect to reach sub-micrometer resolution in the very near future.

Toward Single Fiber Diffraction of Spider Dragline Silk from *Nephila cavipes*

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Efforts are underway to elucidate the origin of the combination of tensile strength and energy absorbing capacity of spider dragline silk. Both the peptide sequence of the proteins and the processing of the silk as it is spun from the spider are thought to contribute to the material's unique properties. To better understand the processing of the silk, an X-ray study of its microstructure as developed during spinning is underway. The results of a preliminary synchrotron X-ray study of the spider dragline silk from *Nephila clavipes* are presented. Comparisons of results from a phosphor storage plate detector system and a image intensified CCD detector are made. The diffraction from 100-fiber bundles was recorded as a function of environment and load on the samples. While the introduction of a dehydrating helium environment affected the tension of the samples, no concurrent change in the scattering behavior was observed. Mechanical loading of the bundles resulted in an increase in the orientation of the scattering centers within the fibers. Ideas for potential experimental protocols for single fiber diffraction are discussed.

Fabrication and Optical Properties of Ultranarrow $In_{0.53}Ga_{0.47}As/InP$ Quantum Wires

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We have fabricated ultranarrow high quality InGaAs/InP quantum wires by high resolution electron beam lithography and deep wet chemical etching. A 200kV transmission electron microscope with a scanning unit and an external pattern generator was used to define ultra small wire patterns on PMMA resist. After the evaporation of a gold layer and a lift-off process we obtained gold masks with lateral widths down to 15nm. Etched wire structures with widths down to 8nm could be realized using a $Br_2/HBr/H_2O$ etchant.

The wires were studied optically by means of photoluminescence spectroscopy at temperatures of 2K, 77K and 300K. Due to the high quality of the wet etched structures even the 8nm wide wires are optically active. The weak decrease of the quantum efficiency with decreasing wire width indicates that there is no significant damage at the sidewalls of the wires. This is in contrast to previous studies on dry etched structures.

The emission energy of the smallest wires is significantly blue shifted compared to the emission energy of a quasi two dimensional reference. An energy shift of 85meV is observed for 8nm wires. The energy shift decreases strongly with increasing wire width and vanishes for lateral widths above 60nm. This indicates that there is a steep lateral potential due to the confinement by the InGaAs/vacuum transition at the etched sidewalls. The experimental data agree very well with the calculated transition energies based on a simple model using the geometrical wire width and the band structure parameters of InGaAs.

The photoluminescence of the quantum wires is linearly polarized parallel to the wire axis. The polarization degree increases with decreasing wire width and reaches values of about 60% for 15nm wide wires.

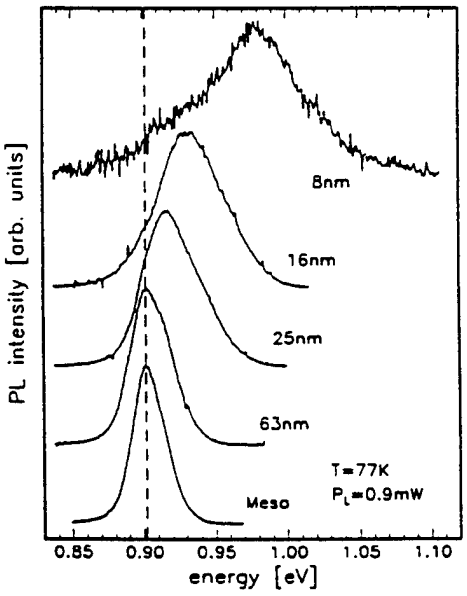

Fig. 1: Photoluminescence spectra of the InGaAs/InP wire structures for different wire widths taken at a temperature of 77K. The intensities are normalized to 1.

DNA Amplification and Detection in a Microfabricated Reaction Chamber

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The application of microfabrication technology to the development of miniaturized analytical instrumentation is an area of active interest and research. As a part of this effort, we are developing a miniaturized thermal cycling device for application to the polymerase chain reaction (PCR). The miniaturization of a PCR thermal cycler and detection system will allow for a portable, low-power, rapid, and highly efficient instrument. This miniaturized instrument will also allow fundamental questions about reaction rates and efficiencies to be answered.

We have successfully amplified several DNA targets from different biological systems in silicon-based microfabricated reaction chambers. These include human immunodeficiency virus (HIV) and ß-globin DNA targets. Previously, we have shown PCR amplification results from single-heater-based, microfabricated, silicon reaction chambers (Northrup et al., 1993). Verification of the target was provided by standard agarose gel electrophoresis (Figure 1). Recent designs include more efficient double-heater reaction chambers. In the present report, will discuss the most recent micro-PCR DNA amplification results, designs and models for reaction chamber optimization, infrared image of reaction chambers, integrated detection concepts, and the overall direction and advantages of the application of microfabrication to DNA-based microinstruments.

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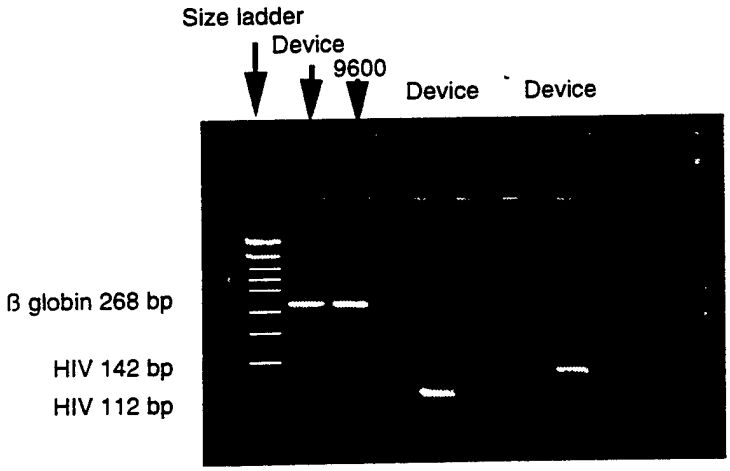

Figure 1. Gel electrophoretic results of micro-fabricated reaction chambers and standard PCR instrument for the amplification of HIV and ß-globin targets.

New Photonic Materials from Genetically Engineered Bacteriorhodopsin

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Bacteriorhodopsin, BR, has attracted the attention of researchers internationally as a novel new photonic material. Unlike present photonic materials it is a material of biological origin. Many investigators have already demonstrated optical processing devices that make use of BR's unusual photochromic, photoelectric and nonlinear optical properties. The unusual photochromic, photoelectric and nonlinear optical properties of this material are a direct consequence of the unique mechanism that has evolved for its biological function. Such a system would be difficult, if not impossible, to make synthetically, and no similar systems exist in the nonbiological world. Recently we have initiated a program, supported by ARO and NSF, aimed at genetically engineering BR to produce a large new family of materials (BR mutants) optimized for device applications. Two major problems are addressed in this program. First, a large number of mutants must be produced and they must be produced at a scale suitable for rapid screening of their photonic properties. This has been done by developing an expression system for synthesizing the mutant BR's in their natural host i.e. *Halobacterium halobium.* The second problem is that present theory is inadequate for accurately predicting changes in the photonic properties of BR when amino acids are altered. Extensive experimental data will be required to develop sufficient understanding so that mutant BR's can be tailored for particular device applications. This requires rapid measurement of photonic properties for different BR mutants using a minimal amount of sample preparation. Three experiments will be described which are used to rapidly evaluate and screen the photonic properties of different BR mutants. These experiments measure the photochromic properties, the photoelectric properties and the nonlinear optical properties of BR mutants. They are novel in that each experiment requires only a small quantity of BR in an aqueous suspension.

Fluorescently Labeled Biological Samples Imaged Under Solution Using a Near-Field Scanning Optical Microscope

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We have constructed a near-field scanning fluorescence microscope designed specifically for imaging fluorescently labeled protein structures in solution at super resolution. The reason for building this new type of microscope is to overcome the resolution-limited conventional optical microscope and the destructive nature of the electron microscope. Our near-field microscope is configured in the transmission mode with the tapered fiber-optic probe as the illuminator and an objective lens as the light collector, located underneath the sample and wet cell. The probe is coated with aluminum, except the very tip, which creates an aperture of $\leq$ 100 nm diameter for light emission. The light emitted from the probe tip remains collimated at the dimension of the aperture for a limited range, called the near-field. Raster scanning of the fluorescently-labeled sample within the near-field region of the probe generates a fluorescent image of super resolution. We have confirmed that fluorescence near-field scanning images can be obtained under solution by imaging charged, fluorescent microspheres adhered to glass. We are attempting to image single skeletal muscle myofibrils whose thin filaments are fluorescently labeled with FITC-phalloidin. The goal is to develop a means of testing recent models of muscle contraction that predict small changes in thin filament length. We hope to be able to detect $\leq$50 nm changes of thin filament length that may accompany transitions between rigor, relaxation, and activation in muscle.

Organizing Proteins at the Membrane Interface *via* Metal Ion Coordination

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Novel lipid interfaces have been formed from copper chelating lipids and natural phospholipids. Lipids containing iminodiacetate chelating polar head groups were synthesized and shown to bind Cu(II) ions in mixed liposomes and monolayers. The binding of histidine rich proteins to the membrane interface was investigated using sedimentation, ESR, and monolayer experiments. Binding constants greater than 2×10^6 M^{-1} were found for myoglobin binding to metal chelating liposomes. The ESR spectrum of chelated Cu(II) on liposomes showed evidence of copper coordination to surface accessible histidines of horse myoglobin shown by changes in spectral line positions upon protein binding. These materials are also being studied for selective orientation of proteins at the interface. Fluorescence studies using pyrene excimer formation within the hydrophobic region of the lipid bilayer was used to investigate multisite attachment of the protein to the interface.

Two-Dimensional Protein Crystals (S-Layers): Fundamentals and Applications

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Crystalline cell surface layers (S-layers) can be found as the outermost cell envelope component in many bacteria. S-layers represent the simplest biological membranes developed during evolution. They are usually composed of homogeneous protein or glycoprotein monomers that are held in a single layer by noncovalent intermolecular bonds. The lattices have either oblique, square or hexagonal symmetry, are generally only 5 to 10 nm thick and exhibit type specific pores of identical size and morphology in the 2 to 8 nm range. S-layer subunits can be removed by hydrogen bond breakage agents from the bacterial cell surface. Upon removal of the desintegrating agent the isolated subunits have the ability to assemble by an entropy driven process into regular arrays in suspension, on solid surfaces (e. g. metals, polymers, glass, carbon, silica), at the air/water interface or on lipid films generated by the Langmuir Blodgett technique. Since S-layers are monomolecular crystalline structures, functional groups are present on the polypeptide or carbohydrate moiety of each constituent subunit in an identical position and orientation. This characteristic feature makes S-layers susceptible for a broad spectrum of chemical modification procedures, leading to specific alterations in the physiochemical characteristics of the lattice in the nanometer range. The unique properties of S-layers led to numerous applications including: (i) immobilization matrices for functional and ordered bonding of molecules (e.g. enzymes, ligands) as required for biosensors, diagnostics, enzyme membranes and affinity microcarriers, (ii) supporting structures for Langmuir-Blodgett films and large scale reconstituted biological membranes which incorporate functional molecules (proton pumps, ion channels, receptor molecules, carriers), (iii) patterning elements in molecular nanotechnology and matrices for manipulating molecules by scanning probe microscopy and (iv) isoporous ultrafiltration membranes.

MANIPULATION AND ASSEMBLING TECHNIQUES FOR FABRICATION OF MICROROBOTIC SYSTEMS

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Abstract

The batch processing techniques used today in micro mechanics are mainly inherited from micro electronics. Even though many beautiful sensor and actuator systems have been realised this way, the methods have the disadvantage of creating essentially two-dimensional structures and systems. In order to build more complex micro systems, new fabrication techniques must be developed. The intention with this poster is to present one approach to accomplish this.

One suggestion of such a complex micro electro mechanical system is a teleoperated micro robot arm intended for applications in medicine/biomedicine/biology, Fig. 1. It would be suited for handling samples of tissue, cells etc. and perhaps also for measuring pressures and concentrations depending on the integrated "smartness" of the system. Other examples of complex microsystems are artificial organs and smart fluidic instruments. A modular approach has been taken using basic building units: actuators, chips containing microelectronics circuits, and solid three-dimensional silicon elements. The only realistic fabrication technique in a short or medium term perspective is micro assembling e.g. placement and subsequent bonding of the microelements.

A large emphasis in this poster is put on the manipulation and bonding technique and on the instrumentation developed for this purpose. The instrument used is a micro manipulator for use *in situ* a Scanning Electron Microscope. This unit is continuously being developed and its current status is four degrees of freedom and micrometer precision. Micro tweezers are used for placement of microelements, and additional processing tools for deposition, machining or heating can be used in the manipulator. One promising bonding technique for silicon micro elements is gold silicon eutectic bonding, which is presently being evaluated. The results of mechanical testing of bonded micro elements show that very strong bonds can be achieved, and that this joining technique can become a useful component in a complete micro assembly scheme.

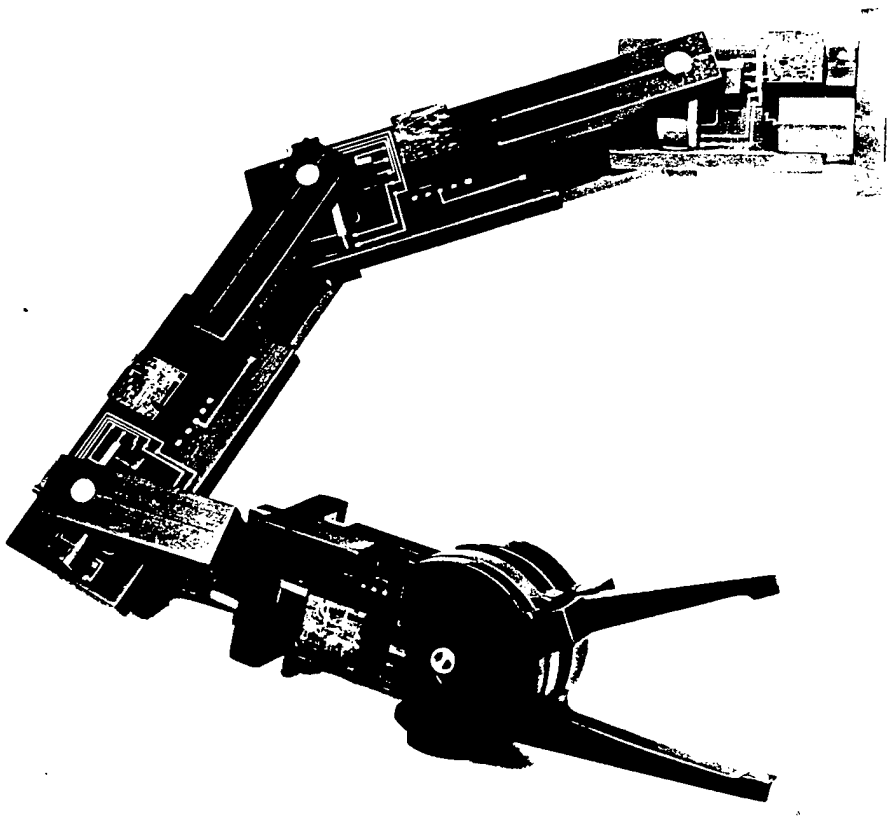

Fig. 1 A design study of a teleoperated microrobot arm (1x1x10 m

DNA Electrophoresis in Microfabricated Arrays

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We will present results of our studies of DNA electrophoresis in microfabricated arrays of silicon obstacles. The ultimate goal of our efforts is to extend the length resolution of DNA electrophoresis beyond the current limit of about 10 Mbp. Microlithography plays an important role in this research for two reasons. First, gels are complex, random, three-dimensional structures making it difficult to understand the motion of DNA. We have used microlithography to construct an array of posts on a square lattice. The posts are 1 μm in diameter, less than 0.5 μm tall and have center-to-center separation of 2 μm. This geometry is far simpler than that of a gel, but it remains a close physical analog. The second reason for using microlithography is the precise control of geometry it affords. Environments can be constructed in which the DNA mobility depends not only on the usual electrophoretic parameters, but on position and direction as well. It should be possible to design geometries tailored to length separation over a particular range of lengths.

Half of our poster will describe observations of DNA in the regular array of posts. The mobility versus length curve will be explained by a model based on the observed dynamics of the DNA. The second half of the poster will describe the behavior of DNA in arrays specifically designed to provide length separation over a certain window of lengths.

Fabrication of Biomolecule-carrying Submicron Structures by Deep-UV and Electron Beam Lithography using *N*-Hydroxysuccinimide Functionalized Perfluorophenyl azides

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We have developed a general method to covalently functionalize polymer surfaces using *N*-hydroxysuccinimide (NHS) functionalized perfluorophenyl azides (PFPAs). The combination of this surface modification technique with deep-UV and electron beam (EB) lithography has produced submicron biomolecular lithographic patterns. Preformed polystyrene (PS) microstructures were spin-coated at 1000 rpm with a 0.5% solution of *N*-succinimidyl 4-azido-2,3,5,6-tetrafluorobenzoate (NHS PFPA ester) in nitromethane and baked at 60 ^{o}C for 20 min. The structures were either photolyzed using a deep-UV lithography contact aligner with a dosage of 10 mJ/cm^2, or exposed with a 15 kV EB with a dosage of 25-75 mC/cm^2. The surface functionalization involves an efficient CH bond insertion into the polymer surface by the highly reactive nitrene intermediate derived from the PFPA upon either deep-UV or EB exposure. In this manner, NHS active ester groups are introduced on the polymer surface and enable the covalent immobilization of amine-containing reagents by way of amide formation. Thus, treatment of the NHS-functionalized lithographic PS structures with *N*-(5-aminopentyl)biotinamide followed by avidin-fluorescein resulted in generation of submicron protein patterns as visualized by fluorescence microscopy. Minimum feature sizes of 0.5 mm and 0.1 mm were resolved for deep-UV and electron beam lithography, respectively.

This surface modification methodology has also been used to functionalize microwaveguides. A 1 mm-size waveguide was fabricated by EB exposure of a resist solution of 5% PS containing 4.6 wt% ethylene 1,2-bis(4-azido-2,3,5,6-tetrafluorobenzoate (a bis-PFPA developed in our laboratory for cross-linking polymers with deep-UV and EB lithography). After spin-coating the NHS PFPA ester solution on the waveguide, only one branch of the waveguide was exposed to an EB. Exposure of the entire waveguide to a solution of 5-(aminoacetamido)fluorescein and subsequent observation under fluorescence microscopy indicated that covalent attachment of the fluorescein to the functionalized branch had taken place. This localized surface functionalization technique may find application in constructing waveguide-based microbiosensors.

The Creation of Biomolecular Arrays by Electrostatic Immobilization on an Insulator Surface Exposed to an Electron Beam

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Surface charging in insulators when they are subjected to irradiation is a known phenomenon. We found that surface charging of insulators by electron beam (EB) irradiation can be used to create biomolecular microstructures. A polystyrene (PS) thin film spin-coated on a silicon wafer was exposed to a 15 kV EB with a dosage of 15-40 mC/cm^2 and treated immediately with a solution of avidin-fluorescein in pH 8.2 buffer. Fluorescent patterns with a minimum feature size of 0.5 mm were observed under fluorescence microscopy. It is likely that EB exposure of the PS film generated volume negative charge which then reacted with the positively-charged avidin-fluorescein, resulting in the attachment of avidin-fluorescein to the PS surface. Another EB-exposed PS film was immersed in a solution of polylysine in pH 8.2 buffer, rinsed and immersed in the avidin-fluorescein solution. "Negative" patterns were observed under fluorescence microscopy, owing to the screening of the trapped negative charge by the positively-charged, non-fluorescent polylysine. A control sample prepared by reacting a PS film with avidin-fluorescein 24 hours after EB irradiation did not show patterns under fluorescence microscopy, consistent with the electrostatic nature of the interaction. Preliminary results showed that avidin-fluorescein microstructures could also be generated in a similar manner on a Teflon film. Since it is possible to generate either volume negative or volume positive charges on insulators, a variety of biomolecules such as polypeptides, proteins (including enzymes), DNA, genes and cells can be immobilized via electrostatic interactions.

THE ENGINEERING FOUNDATION AND FOUNDATION CONFERENCES

The following is a brief summary from The First 75 Years: A History of the Engineering Foundation by Lance E. Metz and Ivan M. Viest.

In 1914, Cleveland manufacturer Ambrose Swasey offered to provide funds for support of engineering research. In response, the United Engineering Society (UES), a public corporation composed of representatives of the societies of electrical, mechanical and mining engineers, established the Engineering Foundation as a department of UES. This new organization was given the power to receive and administer funds from any source for the purpose of supporting "the furtherance of research in science and engineering, or advancement in any other manner of the profession of engineering and the good of mankind."

The Foundation soon began receiving requests for funding various research projects. The first grant was awarded in February 1916 to conduct research on the strength and wearing power of gears. To this day, sponsorship of research projects has continued as one of the principal activities of the Foundation. The Foundation provided financial support to some 400 projects, most of which can be classified as engineering research or engineering-related research.

The first major contribution of the Engineering Foundation to the development of American engineering was the role it played in the establishment of the National Research Council. Created in 1916 by the National Academy of Sciences, at the request of President Wilson for the purpose of engendering cooperation of governmental, educational, industrial and other research agencies in the dual interest of national defense and of scientific and industrial research, the new Council had no funds to begin its operations. The Engineering Foundation filled the need by financing the NRC during the first year of its existence.

However, the bulk of the Foundation's activities through 1930 can be characterized as sponsorship of engineering research projects. Fatigue of metals, thermal properties of steam, investigation of arch dams, concrete and reinforced concrete arches, blast furnace slag investigation, dielectric absorption and electric insulation, earth and foundations, barodynamic research, and the effect of temperature on properties of metals are a few engineering research studies of the 45 projects for which financial support was provided by the Foundation during this period. Mental hygiene of industry, industrial personnel problems, and engineering aptitudes are examples of Foundation-sponsored engineering related studies of this period.

The Fatigue of Metals Project was carried out jointly with the National Research Council. It was the first systematic study of the strength of metals under repeated loading undertaken in the United States. It led to the development of the American Welding Society's welding code that became a basis for the design of machinery, rolling stock, bridges, airplanes, spacecraft, electrical equipment and various other welded devices which were subjected to frequently repeated loads.

The Thermal Properties of Steam Project provided the first authoritative international tables relating steam pressure, temperature and volume in a form readily usable in the design of boilers, engines and other steam appliances. The Arch Dam investigation involved many investigators and is considered a testing ground for research through cooperative efforts.

The Engineering Foundation began its involvement in efforts to improve engineering education through its support of the Engineers Council for Professional Development, known today as the Accreditation Board for Engineering and Technology. To help alleviate the impact of the Depression on individual engineers, the Engineering Foundation sponsored courses for unemployed engineers.

Research on the alloys of iron was the major project which involved cooperative research and writing similar to the activities which were pioneered by the Arch Dam investigation. However, it went a giant step further through its truly national scope and world-wide collection of data. The results were published in a series of 16 books that contained the most encompassing and authoritative information on the subject of steel.

The Welding Research Committee (now Council) was founded in 1935 as an outgrowth of the earlier study of fatigue of metals and was the model for the formation of several research councils during the post World War II period. The WRC successfully promoted the development and use of good welding practices and thus speeded up the acceptance of this basic metal connecting technique.

Between 1946 and 1960 several additional research councils were formed. These independent bodies were formed on

the basis of nationwide representation from academic, industrial, and governmental organizations which were interested in the same broad field of engineering. The Structural Stability Research Council, Research Council on Structural Connections, and Reinforced Concrete Council are three such examples. The research councils planned needed research projects, secured the needed financing, selected the research institution, monitored or supervised the conduct of research and channeled the research findings into practical use. The building of record-height buildings such as the World Trade Center, the Sears Tower, the John Hancock Tower, and various more sophisticated structures such as the Assembly building at Cape Canavarel were made possible only because of the expansion of basic knowledge through the activities of these research councils.

Another major Engineering Foundation contribution was its efforts in the early 1960s to establish the National Academy of Engineering. Through its broad perspective, organizational know-how, and the use of its funds, the Foundation served as a catalyst in the creation of this important institution.

The Engineering Foundation's primary focus during 1961 through 1975 was the establishment of Engineering Foundation conferences. Engineering Foundation conferences were initiated to promote discussions of new knowledge in engineering fields and to develop an interface with other disciplines. The conference format promotes discussion and enhances rapport among participants. These conferences have been instrumental in generating ideas and disseminating information in greater depth than is possible through most conventional presentations or publications. Approximately 25 conferences are held each year.

The conferences sponsored by the Engineering Foundation can be classified into four broad categories:

1. New Interdisciplinary Conferences - Conferences organized on a specific topic of current interest which involves a broad spectrum of the scientific and engineering community. Professional societies may have offered a session in one aspect of the topic at one of their traditional technical meetings. The successful of these conferences depends on the ability of the Chair and Co-Chair to draw conference participants from a wide range of disciplines to discuss the topic in detail.
2. Continuing Interdisciplinary Conferences - Many of the successful new conferences give rise to one or more successor conferences on the same topic which are held on a one to three year schedule.
3. Technical Conferences - Conferences which deal with a specific topic which involves a single general research area and which may be co-sponsored by one or more of the professional societies.
4. Support Conferences - Conferences for which the Engineering Foundation serves as an umbrella support organization for one or more technical groups.

Over 600 such conferences have been held on topics ranging from Corrosion Engineering to Professional Liability to Engineering in Medicine. The Engineering Foundation Conferences Committee is responsible for the planning, management, operation and evaluation of the conferences. Conferences are expected to be self-supporting on a yearly basis, but exact adherence to this goal is considered secondary to the objectives expressed above.

The Foundation's Director and staff provide administrative direction to the conference both from the New York office and at the various sites. The Director and staff are constantly engaged with the conference chairpeople, organizing committees, personnel at conference sites, and participants in their endeavor to make the conferences vital, rewarding and enjoyable.

Through its conferences and research grants, the Foundation became a pioneer in focusing the attention of the engineering community on such emerging areas of concern as urban mass transportation, pollution of the environment, and the technology gap between the Third World and the developed nations.

In 1976 the Engineering Foundation established its Engineering Research Initiation Grants (ERIG) program to support research by new faculty members in technical areas of interest to the Founder societies. It was designed not only to obtain solutions to technical problems but also to help attract and keep talented individuals on the academic staffs of university faculties. These grants also promoted early transfer of research findings to the engineering profession. Since its inception, this program has been characterized consistently by large numbers of high quality research proposals in each of the five major areas of engineering. As of 1990, almost all of the Foundation's discretionary income was channeled into engineering research through the enthusiastically received ERIG program.

Altogether, some 230 leading engineers served as members of the Engineering Foundation Board. They were appointed as representatives of the Founder Societies (ASCE, AIME, ASME, IEEE, and AIChE), or of the United Engineering Trustees and its predecessors, or as members at large. Their broad experiences and foresight resulted in judicious selection of projects and grant recipients, thus multiplying the effectiveness of the whole enterprise.

Founded by a visionary engineer-entrepreneur and guided by successive boards and directors of the highest caliber, the Engineering Foundation has been at the forefront of engineering research for 75 years. By building on this legacy, it is poised to continue to play a catalytic role in the evolution of American science and technology.

Operations of the Foundation are overseen by a Board representing membership from the five Founder Societies of the UET.

Engineering Foundation Conferences

NANOFABRICATION & BIOSYSTEMS: FRONTIERS AND CHALLENGES

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May 08 to May 12, 1994

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